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Assessment of Histological Alterations Induced in The Liver and Kidney Tissues of Adult Mice Exposed to Heat Stress and Their Association with Global Warming

تقييم التغيرات النسيجية المستحثة في أنسجة الكبد والكلى لدى الفئران البالغة المعرضة للإجهاد الحراري وارتباطها بظاهرة الاحتباس الحراري

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Abstract

Background: Our planet's temperature is rising at an alarming rate, threatening the well-being of all life on Earth. This warming disrupts the growth, health, and overall productivity of humans, animals, and ecosystems. **Objectives**: The current study aimed to evaluate the histological alterations triggered in the liver and kidney tissues of adult mice exposed to heat stress. **Methods**: 15 mice were randomly divided into control group (5 mice) which were housed under standard laboratory conditions with a constant temperature of 25°C and experimental group which were divided into two subgroups; heat stress group (5 mice) where mice were exposed to a high temperature of 38°C and severe heat stress group (5 mice) where mice were subjected to an even higher temperature of 48°C. **Results**: Myriad histopathological changes were revealed in the liver and kidney tissues of heat stressed animals including destruction of the hepatic and renal normal structure represented by damaged cells possessing vacuoles in their cytoplasm and altered shaped nuclei which displayed signs of death, either pyknosis, karyorrhexis or karyolysis. Besides, dilatation and congestion of blood vessels. **Conclusion**: The current study confirms that even moderate or severe heat stress can damage the liver and kidneys, potentially leading to death. This aligns with what we know about the dangers of heatstroke. Rising global temperatures pose a significant threat to the well-being of all life on Earth. Heat stress can harm not only humans but also animals and ecosystems.

Key Words: Heat stress, Mammals, Liver, Kidney, Histology

1. Introduction

Our planet is experiencing a rise in temperature, and human actions are a significant contributing factor. Understanding the causes, consequences, and intricacies of global warming is crucial. This knowledge empowers us to act and ensure the well-being of Earth. The gradual rise in Earth's average temperature, mostly brought on by human activity since the Industrial Revolution, is what we call global warming. It's caused by a rise in greenhouse gas levels, like carbon dioxide, trapping heat in the atmosphere (Friedlingstein *et al.*, 2022).

The sustained increase in the mean temperature of Earth has accelerated in the last century. This phenomenon, known as global warming, is largely attributed to the increased burning of fossil fuels. Our growing population has led to a surge in burning fossil fuels like oil, coal, and natural gas. As a result, gases that trap heat are released into the atmosphere, causing a phenomenon called the "greenhouse effect". Imagine Earth like a giant greenhouse. Sunlight warms the planet's surface, but some of that heat tries to escape back into space. Greenhouse gases, like carbon dioxide, methane, and water vapor, act like a blanket, trapping that heat and causing the planet to warm up (Nunez, 2019).

Our planet's temperature is rising at an alarming rate, threatening the well-being of all life on Earth. This warming disrupts the growth, health, and overall productivity of humans, animals, and ecosystems. As Earth heats up, many animals are feeling the strain. The extra heat weakens their immune systems, making them more likely to get sick. This can affect everything from how well they fight off infections to how healthy their babies are. Intense heat harms livestock production, causing substantial economic losses. When the environment gets too hot, animals struggle to regulate their body temperature. This heat stress disrupts their normal bodily functions, significantly reducing their productivity (Cooper and Washburn, 1998) .

The severity of heat stress in animals is directly correlated with the time-frequency of heat exposure (Xie et al., 2014). The thermal neutral zone, also known as the comfort zone, refers to the range of environmental temperatures within which organisms can maintain their core body temperature through minimal physiological adjustments, primarily thermoregulation. Once the air temperature climbs above 25°C, things start to go awry. Animals (and humans!) struggle to maintain a healthy body temperature because their ability to release heat gets thrown off balance. This condition, called heat stress, is a serious threat to our health and can cause big problems for farms and hospitals. It leads to significant economic losses as livestock production suffers and healthcare services are strained (Thornton *et al.*, 2022).

2. The Theoretical Framework

Numerous scientific investigations have documented the impact of severe heat stress on various bodily systems. Research suggests that nearly all organs within the human body are susceptible to its detrimental effects (Helman and Habal, 2007; Hoppe *et al.*, 2008). Heat stress initially impacts the skin, triggering an increase in blood flow to the cutaneous vascular bed (skin surface) through reflex vasodilation. This is accompanied by vasoconstriction in the hepato-splanchnic vascular zone (abdominal organs) to prioritize blood flow redistribution and enhance heat dissipation. The human body initiates thermoregulatory responses, subsequent sweating promotes evaporative cooling, leading to a decrease in core body temperature. Heatstroke represents the most severe health consequence of elevated ambient temperatures. It manifests when the body's thermoregulatory mechanisms fail, leading to a rapid rise in core temperature, disruption of sweat production, and a physiological crisis. Heatstroke presents a constellation of symptoms, including a dangerously high body temperature, dry and hot skin despite no sweating, a rapid heartbeat, and dizziness. These symptoms require immediate medical attention. Heatstroke isn't the only danger zone. Heat exhaustion comes first, with symptoms like heavy sweating, rapid breathing, and a fast pulse. It's a warning sign to cool down before it progresses to heatstroke. Another heat-related issue is muscle cramps, often caused by exercise or exertion in hot weather. Finally, excessive sweating can also lead to heat rash, an itchy skin irritation (Helman and Habal, 2007) .

Research highlights the multifaceted interplay between extreme ambient temperatures and adverse mental health outcomes. Elevated temperatures have been associated with increased rates of suicide, aggression, and violent crime. Studies in the US and Mexico, for example, demonstrate a potential association between a 1°C rise in average temperature and a 1% increase in suicide cases. The disruption of serotonin production, a key neurotransmitter for mood regulation, by heat stress may be a contributing factor (Bundo *et al.*, 2023).

Rising nocturnal temperatures are demonstrably linked to sleep quality deterioration. A recent study estimates a current average annual sleep loss of 44 hours attributable to this environmental factor, with projections indicating further sleep duration reductions in the coming years. Insufficient sleep is associated with a multitude of detrimental health consequences. These include compromised immune function, impaired cardiovascular health, and heightened susceptibility to inflammatory processes and chronic disease development (Minor *et al.*, 2022).

Heat stress poses a significant threat around the world, particularly in hot regions, demanding a complex physiological adaptation across multiple organ systems, including the endocrine, cardiorespiratory, and immune systems (Altan *et al.*, 2003) .

Hyperthermia poses a significant threat to animal well-being. It disrupts reproductive organs, hindering growth, reproduction, and immune function. This results in decreased libido, fertility issues, and lower chances of embryo survival (Kumar *et al.*, 2011). Heat stress disrupts spermatogenesis in male animals, leading to an increase in morphologically abnormal sperm due to testicular injury and reduce male fertility potential (Jung *et al.*, 2001; Kastelic *et al.*, 2019). Also, the ovaries are particularly sensitive to heat stress, which can hinder an animal's ability to reproduce (Hansen, 2009).

Heat stress presents a significant challenge to immune system homeostasis in living organisms. While acute exposure may exhibit a stimulatory effect, chronic heat stress can have an inhibitory role on the immune system's ability to maintain a healthy state (Bagath *et al.*, 2019). Research suggests that high temperatures disrupt the mouse immune system by causing an imbalance in cytokine production which are signalling molecules involved in the immune response (Ayo *et al.*, 2011).

Heat stress affects gastrointestinal tract as it is interrupted especially in children during summer which can influence the gastrointestinal function resulting in diarrhoea. In animals exposed to heat stress there is evidence of change in intestinal absorption of amino acids. However, little is known about the effect of heat stress on gastrointestinal motility (Sengupta and Sharma, 1993) .

Heatstroke is a major threat to the liver, one of the body's most vital organs. Studies using mice as models have shown that heat stress triggers an inflammatory response in the liver, marked by an increase in inflammatory macrophages and the formation of scar tissue (fibrosis) (Roncal-Jimenez *et al.*, 2018). When exposed to high temperatures, the body restricts blood flow to the liver and digestive organs (hepato-splanchnic vasoconstriction). This in blood flow lowers the oxygen reduction reaching the liver and significantly increases its glucose output. These combined effects likely lead to a state of oxygen deprivation (hypoxia) in the liver and digestive system (hepato-splanchnic region). As seen in human and animal studies, hepato-splanchnic hypoxia is known to cause damage to liver cells (hepatocellular damage). This damage is often accompanied by elevated levels of enzymes in the bloodstream, including alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and lactic acid dehydrogenase, as well as creatine phosphokinase, as reported in human (Alzeer *et al.,* 1997) and animal studies (Sharma, 1997; Agrawal and Gupta, 2013).

Kidneys are essential organs that maintain a stable internal environment within the body. When animals are exposed to excessive heat, their kidneys show visible signs of damage. These signs, observed during a general examination, include increased size, a pale and moist appearance, and changes on both sides of the kidneys. Histological examination under a microscope reveals damage to the tiny tubules responsible for filtering waste products from

the blood (acute tubular necrosis). This damage is unevenly distributed (asymmetrically severe) and includes cell death, regeneration of the lining of the tubules (tubular epithelial regeneration), the presence of immune cells (neutrophil influx), and a basophilic appearance of the cells (cellular basophilia). These findings suggest that heat can damage the kidneys (Sula *et al.*, 2012).

In the current investigation, adult albino mice were repeatedly subjected to moderate and severe heat stress. The liver and kidney histological and morphological abnormalities were noted.

3. Methods of Research and the tools used

3.1. Experimental Animals

Fifteen adult male mice (Mus musculus) weighing between 20 and 25 grams were breed in the animal house located in Theodor Bilharz Research Institute, in El-Giza, Egypt. They were kept in two-animal transparent plastic cages with wood chips for bedding in a climate set to 25 °C with a 55.5% relative humidity and a 12-hour light/dark cycle. Ad libitum access to tap water and standard laboratory mouse food was offered. The mice were given a week to become used to their new environment.

3.2. Animal Housing and Treatment

The mice were split into two groups: five animals for the control group and ten for the heat-stressed group .

The control group: These mice were housed under standard laboratory conditions with a constant temperature of 25°C±0.5°C and had free access to water throughout the experiment.

The heat-stressed group: The mice were subdivided into two subgroups of 5 animals each; moderate heat-stressed group where these mice were exposed to a moderate temperature of 40°C for 1 hour per day in a controlled incubator for 7 consecutive days and severe heat-stressed group where the mice were subjected to an even higher temperature of 48°C for 1 hour daily in the incubator for 7 days. All animals had ad libitum access to water and food (Datta, 2001).

3.3. Histological Preparations

Small pieces of the liver and kidney of all experimental mice were preserved rapidly in Bouin's fixative for a full day. Then, they were subjected to the normal procedures for paraffin sectioning. After that, they underwent the standard protocols for paraffin sectioning. Following standard processing, hematoxylin-eosin (H&E)-stained 4 µm slices were dehydrated in escalating ethyl alcohol series, cleaned in xylene, and mounted in DPX (Bancroft and Gamble, 2002). A light microscope was used to view the stained

sections, and when necessary, photomicrographs were taken.

4. Results of Research

4.1. Morphological observations

The mice of the control group, housed at a comfortable 25°C, exhibited no abnormalities. Whereas the mice exposed to moderate heat stress (38°C) tolerated the temperature and survived. However, they showed signs of distress, including decreased activity compared to their normal behaviour and darkening of the fur around their necks (Figure A). Unfortunately, mice subjected to severe heat stress (48°C) couldn't cope for long. Initially, they displayed resistance behaviours, but most succumbed to the extreme temperature

Figure (1): Heat-stressed mice exhibited darkening in the neck area (indicated by the black arrows)

4.2. Histological results

4.2.1. The Liver

Normal mouse livers, as shown in **Figure (2a&b)**, are organized into structures called hepatic lobules. These lobules are separated by thin layers of connective tissue, forming compartments. Within these compartments, called portal spaces, lie tiny branches of three important structures: the portal vein, the hepatic artery, and a bile ductule. Each lobule also has a central vein running through its core. Inside each lobule, sheets of liver cells, called hepatocytes, spread outward from the central vein. Sandwiched between these sheets are tiny blood channels known as sinusoids. These sinusoids are lined by a very thin layer of specialized cells called sinusoidal endothelial cells which are flat and have fenestrations that allow the exchange of materials between the blood and the liver cells. Large scavenger cells, called Kupffer cells, are also present. These Kupffer cells have large, dark nuclei and reside within the cavities of the sinusoids, acting as a defense system against harmful pathogens. Finally, tiny channels called bile canaliculi weave between the rows of liver cells. The fundamental building blocks of the liver lobule are hepatocytes. These polygonal-shaped cells, with one or two round nuclei are the champions when it comes to the liver's chemical processes. They orchestrate most of the synthetic and metabolic functions happening within the lobules. Interestingly, hepatocytes have an affinity for basic dyes, making them readily visible under a microscope, appearing quite stained.

Livers from heat-stressed mice (**Fig. 2c,d,e&f**) showed dramatic histological changes. The normally organized lobules lost their structure, and most hepatocytes appeared damaged. These damaged cells had vacuoles in their cytoplasm and altered shaped nuclei which displayed signs of death, either pyknosis or karyorrhexis. The damage wasn't limited to the liver cells. The central veins, portal arteries and portal veins were also sharply affected. They appeared dilated and clogged with blood masses. Worse yet, inflammatory cells, typically involved in inflammation, were seen invading the areas around these vessels. The endothelium of these blood vessels appeared ruptured in some places. Even the sinusoids within the lobules, called were devastated and were congested with stagnant blood and lined by enlarged Kupffer cells which detached from the walls of the sinusoids.

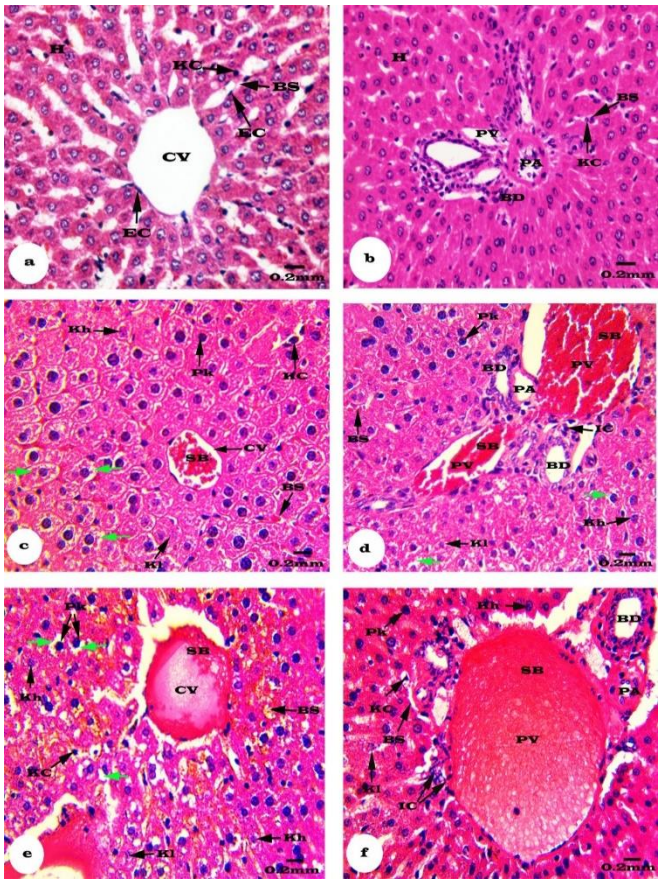

Figure (2). Light microscope images (a-f) of the centrilobular and periportal zones of liver tissue of mice stained with hematoxylin and eosin (H&E) showing (a&b) control hepatic tissue appeared with a rounded central vein (CV), surrounding with cords of healthy hepatocytes (H) which are separated by thin blood sinusoids (BS) lined with regular Kupffer (KC) and endothelial (EC) cells. Besides, intact portal zone containing branches of portal artery (PA), portal vein (PV), and bile ductule (BD) was seen; (c&d) liver tissue from moderately heat-stressed mice illustrating destructed portal vein (PV), portal artery (PA), and central vein (CV) which were enlarged and congested with stagnant blood (SB). The hepatocytes seemed distorted having vacuolated cytoplasm (green arrows) and distorted nuclei appeared pyknotic (Pk), karyorrhexed, (Kh), or karyolysed (Kl). The blood sinusoids (BS) appeared congested and lined with rounded and enlarged Kupffer cells (KC); (e&f) liver tissue from severely heat-stressed mice displayed extensive damage where most of the hepatocytes revealed clear signs of cytoplasmic vacuolation (green arrows) and nuclear pyknosis (Pk), karyorrhexis (Kh), and karyolysis (Kl). The lining of the blood sinusoids (BS) appeared abnormal, with more rounded Kupffer cells (KC). The central vein (CV), as well as the portal vein (PV) and portal artery (PA), were all widened and congested with stagnant blood (SB). Additionally, inflammatory cells (IC) were seen invading the tissue.

4.2.2. The Kidney

Slices of kidney tissue from control mice (**Fig. 3a&b**) showed a normal histological structure. The outermost layer, called the capsule, appeared well-organized. Below this capsule were two main regions: the cortex (outer region) and the medulla (inner region). Within the cortex, numerous round structures called Malpighian corpuscles were visible. Each Malpighian corpuscle is made up of two layers, forming a capsule around a cluster of tiny blood vessels, called the glomerulus. Also in the cortex, many small proximal convoluted tubules were seen having a narrow lumen and lined with a single layer of cube-shaped epithelial cells

possessing free ends have a fuzzy brush-like border, and their cytoplasm stains dark with a basophilic dye, besides centrally rounded nuclei. The cortex also contains larger distal convoluted tubules lined with cube-shaped cells, but they have more nuclei visible per slice. The renal medulla houses the loop of Henle (the descending and ascending limbs), along with collecting tubules. The descending limb features very thin walls lined by flattened squamous epithelium, enclosing a relatively wide lumen. These cells exhibit uniformly eosinophilic cytoplasm with nuclei bulging inwards. In contrast, the ascending limb has thicker walls lined by cuboidal epithelium and a narrow lumen. The cytoplasm in these cells is also generally eosinophilic, but with less distinct cell borders. These cells have prominent, centrally located nuclei that stain deeply basophilic. The collecting tubules are lined by simple cuboidal epithelium that transitions to taller cells distally. Their cytoplasm stains weakly and contains large, centrally located oval nuclei.

Kidneys from moderately heat-stressed mice (**Fig. 3c&d**) showed clear signs of histological damage. The cortex contained damaged Malpighian corpuscles, which appeared with narrowed Bowman's space and expanded glomeruli showed mesangial hypercellularity. Also, the proximal and distal convoluted tubules were damaged showing signs of focal tubular necrosis and contained a substance called hyaline casts or cellular debris in their lumens. The lining cells of these tubules also showed signs of cell death, including nuclear pyknosis and karyorrhexis. Furthermore, the blood vessels between the tubules appeared severely damaged and contained stagnant blood. The medulla showed significant damage represented in the disfigure of the lining epithelium of the loops of Henle (descending and ascending limbs) of and the collecting tubules as shown in **Figure (3c&d)**.

Severe heat stress caused significant pathological alterations in the kidneys of mice (**Fig. 3e&f**). Renal corpuscles displayed glomerular hypertrophy with increased Bowman's capsule spaces. The convoluted tubules exhibited pyknotic or karyorrhectic nuclei, indicative of cellular injury. The renal medulla showed marked irregularities in the epithelial linings of the loops of Henle (the descending and ascending limps) and collecting tubules which revealed nuclear pyknosis. Moreover, the intratubular gaps were congested with blood.

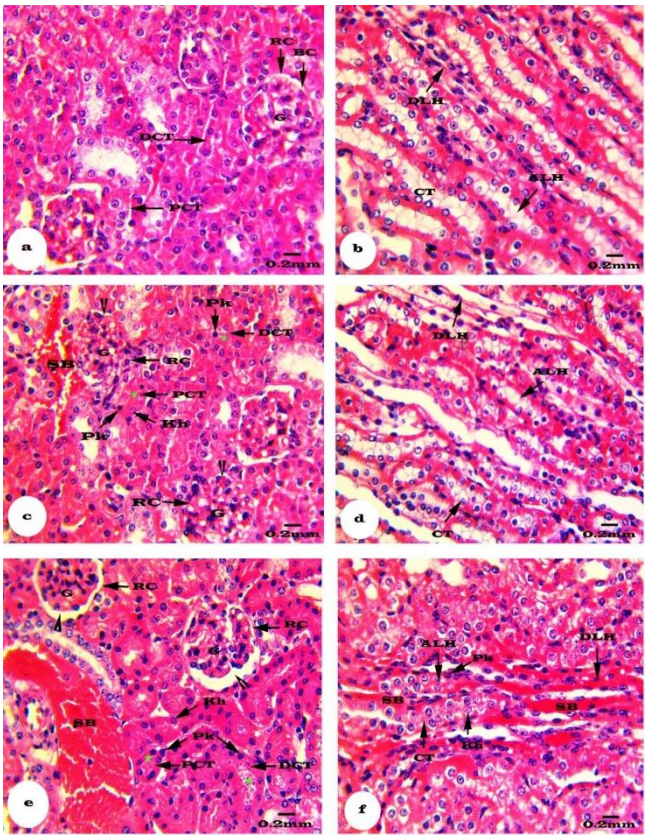

Figure (3): Light microscope images (a-f) of the renal tissues of mice stained with hematoxylin and eosin (H&E) showing (a&b) control renal tissues appeared with intact renal corpuscles (RC) having regular Bowman`s capsules (BC) and glomeruli (G), besides the convoluted tubules (the proximal (PCT) and distal (DCT)) in cortical area, also regular loops of Henle (the descending (DLH) and ascending (ALH) limps), and collecting tubules (CT) in medullary area of control mice; (c&d) damaged renal cortical portion showing altered renal corpuscles (RC) with decreased Bowman's spaces (arrowheads) and expanded glomeruli (G), as well as tubular necrosis of the convoluted tubules; proximal (PCT) and distal (DCT) which having cellular debris (green asterisks), and showed nuclear pyknosis (Pk) or karyorrhexis (Kh) of their epithelia were seen. The renal medulla with conspicuous disfigured lining of the descending (DLH) and ascending (ALH) limbs of Henle loop, and collecting tubules (CT), beside congested blood vessels with stagnant blood (SB) were noticed in moderate heat stressed mice; (e&f) deteriorated renal cortex with malformed Malpighian corpuscles showing hypertrophied glomeruli (G) and widened urinary spaces (arrowheads), besides the proximal (PCT) and distal (DCT) renal tubules were filled with hyaline casts (green asterisks) and having pyknotic (Pk) or karyorrhexed (Kh) nuclei. Also, destructed renal medulla showing stagnant blood (SB) in the inter-tubular blood vessels, and nuclear pyknosis (Pk) of the lining epithelia of both limbs of the loops of Henle; the descending (DLH) and ascending (ALH), and the collecting tubules (CT) were seen in severe heat- stressed mice.

5. Interpretation of Results

Heat damage can cause various changes in the microscopic structure of different organs. The present investigation aimed to evaluate the histological alterations triggered in the liver and kidney tissues of adult mice subjected to heat stress. The liver is an extraordinary organ which performs a multitude of functions, such as secreting bile, breaking down bilirubin, breaking down proteins, lipids, and carbs, detoxifying all metabolic agents, and storing minerals and vitamins. Along with secreting angiotensinogen and activating vitamin D, thyroxin (T4) is changed into triiodothyronine (T3). Moreover, the liver uses Kupffer cells to phagocytose, eliminate haemolysis products, and filter portal blood (Ozougwu, 2017).

The present study's findings showed that the livers of heat-stressed mice exhibited a variety of histological changes. These changes included congestion and dilation of the central and portal veins with stagnant blood masses in their lumens, congestion, and dilation of the hepatic blood sinusoids with activated Kupffer cells and damaged endothelial cells, besides severe accumulation of infiltrated inflammatory cells. Furthermore, the bulk of the hepatocytes showed obvious symptoms of necrosis and degeneration, and the hepatic strands looked detached.

The liver is very sensitive to high temperature. When animals are exposed to heat stress, the portal vein bringing blood to their livers has a higher level of damaging molecules. This can starve the liver cells of oxygen and make them stressed (Hall *et al.*, 1999). Heat stress may seriously harm the liver, as seen by the degenerative changes that occur in the liver parenchyma following exposure to heat. These results closely resemble those noted by Sharma (1997).

Heat stress triggers vasodilation, leading to selective hyper perfusion in the periphery. This shunts blood flow away from visceral organs to

maximize heat dissipation (Deja *et al.*, 2010). During hyperthermia, the liver's blood supply behaved differently from the rest of the body. Even when the heart pumped twice for much blood (doubled cardiac output), blood flow to the liver decreased significantly. This points towards ongoing dilation of blood vessels in the periphery, a common response to radiant heat (Deja *et al.*, 2010). Histological examination of centrilobular, intralobular, and biliary vessels revealed dilatation when the animals were gradually brought up to 40.5°C for their core body temperature in an incubator (Vlad *et al.*, 2010). Whole-body hyperthermia in rats induces profound systemic and local vasodilation, potentially leading to a significant decrease in hepatic microvascular blood flow, approaching a state of stasis. This reduction in flow can result in hepatic tissue hypoxia and subsequent cellular damage (Vlad *et al.*, 2010).

Also, hyperthermia induced by a 42°C heat chamber in mice resulted in hypoxia-induced cellular injury. This phenomenon is attributed to a decrease in circulating antithrombin, leading to increased thrombotic responses within the vasculature. The combination of these factors, including vascular stasis, culminated in enhanced thrombin generation and subsequent cellular ischemia (Hernández-Espinosa *et al.*, 2007). Elevated temperatures lead to an increase in free energy within the cellular environment. This additional energy can disrupt the stable folded conformation of proteins by weakening the intramolecular hydrogen bonds. This process, known as protein denaturation, can have deleterious consequences for cellular function. In response to heat stress, the transcription and, in some cases, the oligomerization of heat shock proteins are induced. These molecular chaperones serve a crucial role in mitigating the detrimental effects of hyperthermia by assisting with protein folding, refolding, and preventing protein aggregation (Bouchama and Knochel, 2002). If the heat stress is overwhelming or the body's heat shock protein response is insufficient, the resulting protein damage can go beyond simple unfolding. This has been observed in the livers of mice exposed to high temperatures (Yan *et al.*, 2006).

Hyperthermia not only disrupts protein structure but can also interfere with many other essential cellular processes, potentially leading to cell death. In humans, cell death from heatstroke is a risk when body temperature surpasses 41°C (Hildebrandt *et al.*, 2002) and can even be fatal if core body temperature goes above 42°C (Liechti, 2014). While a certain level of liver damage is a frequent manifestation of heat stroke (>39°C), mortality is seldom a direct consequence of the severity of hepatocellular damage (Deja *et al.*, 2010, Jin *et al.*, 2012). Isolated mouse hepatocytes exposed to heat stress (40.5°C) exhibit a decrease in cell viability of up to 35%. This phenomenon is putatively attributed to a multitude of cellular impairments, including cell membrane instability, mitochondrial dysfunction, and compromised protein transport mechanisms (Araújo *et al.*, 2019).

Research investigating the impact of lower hyperthermic temperatures on a particular liver cell populations remains scarce. However,

studies employing a whole-body hyperthermia model in rats (40°C for 4 hours) observed a decrease in the cytotoxic activity of liver sinusoidal mononuclear cells. Furthermore, isolated rat sinusoidal natural killer cells exhibited a decline in cytotoxic activity following in vitro heat stress (41°C for 4 hours), potentially attributable to a disruption in target cell binding (Sitnicka *et al.*, 1993). The decline in cytotoxic activity can be plausibly attributed to the previously established increase in cell membrane fluidity and instability. A recognized indicator of liver damage, Kupffer cell hyperplasia, was seen by Agrawal and Gupta (2013) in their investigation of repetitive heat stress of moderate level. A hyperthermic environment disrupts membrane homeostasis, potentially compromising the proper function of these immune cells (Araújo *et al.*, 2019). Hyperthermic conditions exceeding 41°C exacerbate protein denaturation. This phenomenon disproportionately affects lower abundance proteins, leading to their degradation and subsequent aggregation. The resulting protein aggregates can serve as pro-apoptotic stimuli, triggering programmed cell death pathways. While the cessation of hyperthermia allows for the restoration of most cellular processes, the complete recovery of DNA synthesis and associated growth-related functions may necessitate a more extended timeframe (Walter *et al.*, 2016).

In this study, necrosis was observed in most hepatocytes. It can result from lack of blood supply. Nuclear pyknosis, karyorrhexis, and karyolysis are the three most noticeable symptoms associated with necrosis. Pyknosis is the term for the condensation or shrinkage of chromatin at the outer edge of nuclei. It is also used to describe the irreversible condensation of chromatin components in the nucleus of a hepatocyte or any other cell that is going through apoptosis or necrosis. Next comes karyorrhexis, which is the term for the nucleus' catastrophic disintegration with its chromatin dispersed throughout the cytoplasm. Ultimately, endonucleases enzymes cause chromatin of the necrotic cell to completely dissolve, a process known as karyolysis (Abdelhalim and Jarrar, 2011). In the current investigation, cytoplasmic vacuolation was also documented. This damaging occurrence could be a sign of necrosis, that has been shown in a variety of animal cells upon exposure to numerous pathogens (Huang *et al.*, 2020; Yousef *et al.*, 2022; Aboelwafa *et al.*, 2022a).

The kidneys are particularly susceptible to the negative effects of drugs and chemicals because they actively filter and concentrate a wide range of substances and chemicals that can build up to dangerously high concentrations. This often leads to the production of reactive metabolites, which are recognized as important factors in the pathophysiology of peripheral kidney injury (Evenepoel, 2010). According to the current findings, the renal tissues of treated mice showed significant histological changes as a result of heat stress. These changes were seen in the renal cortex and medulla. The damaged renal corpuscles, the congested glomerular capillary, the tubular degeneration, the inter-tubular haemorrhage, and the inflammatory cells infiltration were the most obvious indicators of a renal deterioration.

Heat stress can significantly impact kidney function, especially during intense or prolonged exposure to high temperatures. When the body increases blood flow to the skin and perspires to cool off, blood flow to the kidneys can be reduced. This can hinder their ability to maintain proper electrolyte balance. Additionally, dehydration caused by excessive sweating can further strain the kidneys. Prolonged or severe heat stress can escalate from reduced blood flow to the kidneys to more serious conditions. Heat exhaustion or heat stroke, caused by extreme heat exposure, can significantly decrease blood flow and oxygen delivery to the kidneys, potentially leading to acute kidney injury. Furthermore, heat stress can be particularly dangerous for individuals with pre-existing kidney problems like chronic kidney disease or renal insufficiency. The reduced blood flow caused by heat stress can worsen their existing condition and potentially lead to kidney damage or even failure (Glaser *et al.*, 2016).

Histological evidence of damage, including vacuolization, severe necrosis, pyknotic nuclei, and degenerative alterations, was observed in the tubular epithelial cells lining the mice under heat stress. There was also desquamation of the damaged cells and shedding in the tubular lumens. Furthermore, there were indications of oxidative stress in a number of distal tubules and collecting ducts. According to Epstein (1997), this part of the nephron is less oxygenated than the proximal tubules, which makes it more vulnerable to oxidative stress.

Hyaline deposits were also seen in the kidney tissues' renal tubules, which may also be a sign of renal damage brought on by an error in the metabolism of proteins. Certain defective renal tubules have lining epithelia that exhibit cytoplasmic vacuolation. A significant influx of water and Na^+ ions due to an abnormality in renal cell membrane function may be the cause of this cytoplasmic vacuolation (Aboelwafa *et al.*, 2022b).

The current results showed that heat-stressed mice exhibited darkening in the neck area. The cause of this darkening isn't entirely clear, it's possible due to vasodilation where heat exposure can cause blood vessels to dilate, increasing blood flow to the skin which could cause the neck area to appear darker; sweating when mice sweat to cool down. If they're sweating more due to heat stress, it could lead to a buildup of moisture and pigments in the fur, causing darkening. Also, skin irritation where heat stress can irritate the skin, leading to inflammation and changes in skin pigmentation.

6. Conclusion

The current investigation established that exposing mice to heat stress caused severe histological changes in the liver and kidney tissues, resulting in hepatic and renal toxicity. The present results represent an alert for the noxious impacts of hyperthermia on the body organs of animals which may endanger their lives.

Further investigations are needed to cover the possible histological changes that could be

occurred in other body organs of animals subjected to heat stress.

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