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Fasting as a Therapeutic Strategy to Alleviate Heat-Induced Liver and Kidney Damage in mice: A Physiological and Histological Investigation

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Abstract

Background: Sugar consumption and rising global temperatures threaten metabolism, causing overheating and liver and kidney damage. Fasting may prevent and reverse physiological stress from high temperatures, environmental stress, and sugar-induced damage. **Materials and Methods:** Pregnant albino mice were randomly assigned to the Control, HTEM, Sugar, and Fasting groups. Five fasting cycles were performed by the Fasting group, which fasted for 48–60 hours before eating for four days. PCNA and WT1 expressions in liver and kidney tissues were measured physiologically, histologically, and immunohistochemically. **Results and Conclusion:** Fasting significantly decreases oxidative stress compared to sugar-based diets and heat stress. Fasting enhances liver and kidney function, antioxidant defense enzyme activity, and histoarchitecture. Fasting groups had lower PCNA protein expressions in hepatic tissue and WT1 expressions in renal tubules than HTEM and Sugar groups. These findings reveal that fasting may lower metabolic strain from high temperatures and sugar consumption, offering novel climate-related health solutions.

Keywords: Environmental stressor; Oxidative stress; Fasting; Thermal stress; Hepatic function; High-glucose diet

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Introduction

Climate warming has detrimental effects on the surviving environment and inflicts major damage on the health of living organisms (1). The escalating focus on the impact of persistent high-temperature conditions on health and relief efforts is gaining momentum (2). An elevated temperature environment triggers a cascade of stress responses in the body, causing the uncertainties of internal metabolism, tissue and organ damage, and exhaustion (3). Maintaining physiological functions, such as cellular biology and enzymatic activities, requires an appropriate body temperature, as the high core temperature can distort intraocular proteins and enzymes, resulting in cell injury (4). Recurrent heat stress can develop chronic kidney disease (CKD), low-grade liver damage, and renal injury in mice. This can lead to hepatic accumulation of inflammatory macrophages, while the kidneys exhibit proximal tubular injury characterized by loss of brush border, low-grade inflammation, and renal fibrosis (5). Metabolic syndrome is closely related to fatty liver and chronic renal disease. Consuming fructose has a comparable effect on the kidneys

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and the liver; rats given a high-fructose diet exhibited enlarged kidneys with higher triglyceride levels compared to control rats (6). Moreover, a diet rich in fat and sugar leads to liver steatosis, lobular inflammation, hepatocyte ballooning, and portal inflammation (7). The heightened liver enzyme activity, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), indicate cholestasis, liver hepatocyte necrosis, hepatocellular diseases, and inflammation (8). The kidneys provide the functions of maintaining homeostasis, regulation, and excretion, and depending on their functionality level, they may exhibit various disorders. The predominant techniques utilized to evaluate renal function are biochemical assays, which entail analyzing serum samples to determine urea concentration levels and creatinine clearance (9). Uric acid is commonly regarded as a simple marker of kidney impairment and is consistently associated with both renal impairment and hypertension (10).

Recently, fasting has emerged as a possible strategy to avoid major dietary changes while obtaining strong effects for one or more disease risk factors associated with metabolic syndrome, cancer, cardiovascular disease, and neurodegenerative diseases (11). Calorie restriction, which involves reducing calorie consumption by 20%–40%, has been found to protect against aging and oxidative stress in many organisms (12). Furthermore, it is regarded as a potent and repeatable measure for enhancing longevity, bolstering resilience to stress, and postponing age-related diseases such as cancer in many species, including mammals, rats, and mice (13). A study has examined two primary types of fasting in rodent models: intermittent fasting (IF) and periodic fasting (PF). The IF involves consuming a few calories or only water for less than 24 hours, followed by a normal feeding period of 1–2 days. However, PF involves abstaining from food for two or more consecutive days, with at least one week between each fasting cycle (14). In rodents, IF enhances cognitive ability, strengthens insulin sensitivity, and reduces heart rate and blood pressure (15).

Meanwhile, the effects of prolonged fasting and PF have been extensively researched in yeast, bacteria, and worms (14), and their impacts on the lifespan and well-being of rodents are currently being investigated. Recently, IF, PF, and time-restricted feeding have emerged as potential methods for avoiding significant dietary alterations by offering many benefits, including disease prevention and enhanced therapy (11). Fasting in mice is a commonly performed method in several types of study, mostly to minimize variability in investigative parameters or facilitate surgical operations (16). Research findings from animal and human research provide substantial evidence to support the idea that fasting, specifically IF, is a dietary pattern that enhances overall well-being (17). The ability of the diet to stop disease development and progression has been extensively examined. An innovative theorem argues that meal times are crucial and have been related to cancer development and metabolic health (18). In mice, IF alters liver metabolism and lowers liver weight, potentially contributing to the health benefits of fasting and providing treatment options for long-term conditions such as non-alcoholic fatty liver disease (19). Further investigations are required to examine the mechanisms via which IF regulates liver metabolism (20).

Accordingly, we hypothesize that fasting plays a beneficial role in improving liver and renal abnormalities caused by hyperthermal stress. Our study aims to examine the preventive properties of PF in mitigating the adverse health consequences induced by heat stress on the liver and kidney tissues of mice. To achieve our goal, we analyzed various characteristics that define the activities of the organs under investigation, including the levels of oxidative stress and inflammation.

Material and methods

Animal selection and care

Adult pregnant female CD1 (Swiss) albino mice (6–12 weeks, 25 ± 3 g) were acquired from the Medical Research Institute, Alexandria University animal facility (Animal procedures were approved by AU10230615301). The animals were maintained in plastic cages with wood shavings as bedding in a controlled environment of a 12:12 h light-dark cycle at 22 ± 2 ºC. Throughout the study period, the mice were provided with a precisely measured laboratory meal and had unrestricted access to fresh tap water for drinking. The day on which the vaginal plug was identified, it was designated as day 0 of pregnancy.

Study design

The mice were divided into two groups: a Control group (n = 5) of neonatal mice born from untreated mothers and an experimental group (n = 4) of pregnant female mice exposed to hyperthermia. Hyperthermia was induced by utilizing an electric heater in a closed room, maintaining a temperature range of 39–41ºC for 12 h a day, from days 10 to 13 of pregnancy, for 5 consecutive days. Following birth, once the offspring (n = 15) reached the age of 3 weeks, the newborn mice were evenly distributed into 3 groups (n = 5) each. The groups in the study were the High-Temperature Exposed Mice (HTEM) group, the Sugar group, and the Fasting group. The HTEM group had unrestricted access to standard food and water; the Sugar group was given a diet with a high concentration of glucose solution added to their normal food; the Fasting group was deprived of food for 48-60 h but had access to water to prevent dehydration. After the fasting period, they were fed normally for four days. This feeding protocol was repeated until the mice reached seven weeks old (21). There were 5 cycles of PF over 4 weeks: In the first cycle, the mice were fasted for 48 h with access only to water, and the next cycles lasted for 60 h each. During fasting, mice were placed in separate cages to reduce cannibalism and coprophagy. The body weight was recorded immediately before and following fasting (21).

Sample preparation and organ collection

The neonate mice in all groups were anesthetized at seven weeks of age, and blood samples were obtained from the trunk using heparin tubes. The liver and both kidneys were collected, washed, promptly immersed in a fixative solution containing 4% paraformaldehyde, and prepared for subsequent histological examination. Our study employed all methodologies and approaches to mitigate any distress experienced throughout the experiments.

Biochemical analyses

Serum ALT and AST activities were measured using commercially available diagnostic laboratory tests (Lachema, Brno, Czech Republic). Colorimetric analysis was conducted utilizing the SEMCO S/E-UV spectrometer, adhering to the procedures outlined in the protocols established by (22).

Determination of renal biomarkers in serum

Serum urea (23), creatinine (24), and uric acid levels (25) were measured using reagent kits acquired from Biosystems (Spain).

Renal and hepatic oxidative stress biomarkers

The functions of the antioxidant system and oxidative stress were assessed by measuring malondialdehyde (MDA) levels in liver and kidney homogenates, which served as a marker for lipid peroxidation. The kidneys and livers from each experimental group were gathered, placed in tubes containing 1.5 mL of saline solution, homogenized, and centrifuged at 10,000 rpm for 10 min at 4 ºC (26). The activities of reduced glutathione (GSH) (27), Superoxide dismutase (SOD) (28), and glutathione-S-transferase (GST) (29) enzymes content was also measured. The total protein content was calculated as mg/g tissue using bovine serum albumin as a standard (30). Catalase (CAT) was measured (31) and expressed in mU/mg protein. The reaction was monitored to measure the decrease in H_2O_2 concentration in the sample. The spectrophotometric measurement was used to determine the absorbance of the reaction mixture,

which consisted of the supernatant, phosphate buffer (50 mM, pH 7.0), and freshly generated H_2O_2 (10 mM), at a 240 nm wavelength.

Histopathological and Immuno-histochemical examination

After fixing liver and kidney tissue samples in a 4% paraformaldehyde solution for 24 h, the samples were washed twice with 1x PBS for 30 min each. The samples were embedded in paraffin, cut into sections measuring 4–5 µm in thickness, stained with hematoxylin and eosin (H&E), and analyzed using light microscopy. For immunohistochemical analysis, epitope retrieval was performed by boiling the samples in a microwave with 10 mM sodium citrate at pH 6.0 for 3 min. Subsequently, the sample was washed using phosphate-buffered saline (PBS) and then treated with a blocking solution consisting of 5% bovine serum albumin in PBS with 0.1% Triton X-100 for 15 min. The proliferating cell nuclear antigen (PCNA) protein (ab218310, Abcam ltd., Cambridge, UK) was detected in liver samples using a primary antibody specific to PCNA, commonly used as a marker for cell proliferation. A primary antibody targeting the Wilms' tumor suppressor gene 1 (WT1; ab224806, Abcam ltd., Cambridge, UK), a crucial protein for proper kidney development, was applied to kidney samples and incubated at 4 °C overnight in a moistened chamber. Appropriate horse radish peroxidase (HRP) conjugated secondary antibodies were employed to detect the primary antibodies.

Statistical analysis

GraphPad Prism 5 software (San Diego, California) was deployed for all statistical analysis, reporting data as means with standard deviation (SD). One-way ANOVA was used to conduct the statistical comparisons, followed by Tukey's test post hoc analysis. *P* values of 0.05 were considered significant differences.

Results

Liver function tests

The analysis of liver function markers demonstrated the impact of consuming a sugar-enriched diet and heat exposure. Our findings indicated a significant increase in liver function in the Sugar and HTEM groups compared with the Control groups. Moreover, fasting resulted in a significant reduction in liver functions compared to mice that were fed an enriched sugar diet (**Figuers 1A–B**).

Hepatic oxidative stress biomarkers

The results demonstrated a statistically significant rise in MDA level in the HTEM and Sugar groups compared to the Control ($P < 0.05$). However, fasting reduced MDA concentration compared to HTEM and Sugar groups.

Antioxidant system

The HTEM and Fasting groups exhibited a significant reduction in CAT, GST, GSH, and SOD expression compared to the control. Conversely, these parameter expressions increased in the Fasting groups compared to the HTEM and Sugar groups (**Figuers 1A–B**).

Kidney function tests

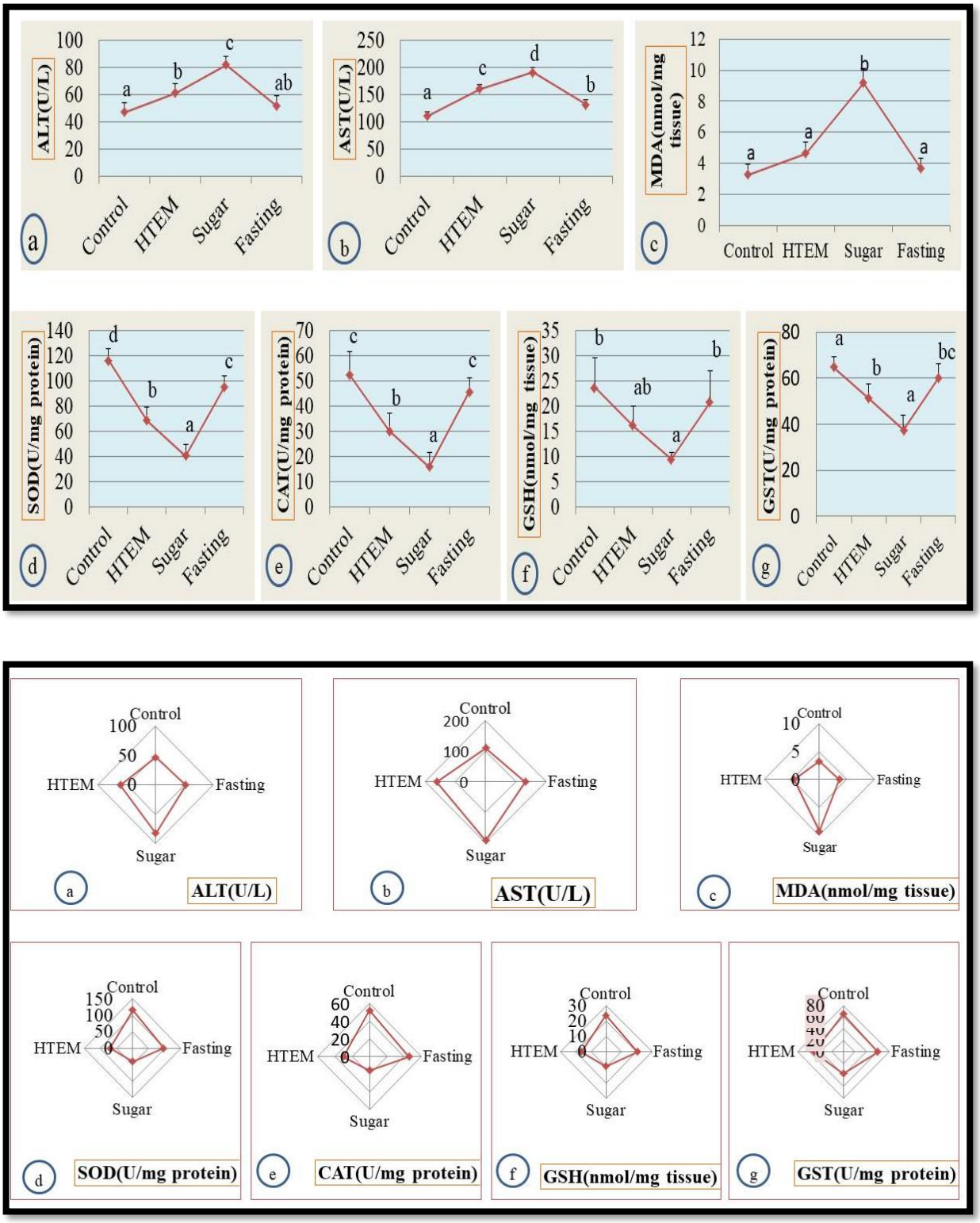
Analyzing kidney function demonstrated the impact of consuming a sugar-rich diet and heat exposure. The findings indicated a significant increase in renal function in the Sugar and HTEM groups compared to the Control groups. However, fasting resulted in a significant reduction in renal function compared to the mice fed a diet rich in sugar (**Figuers 2A–B**).

Renal oxidative stress biomarkers

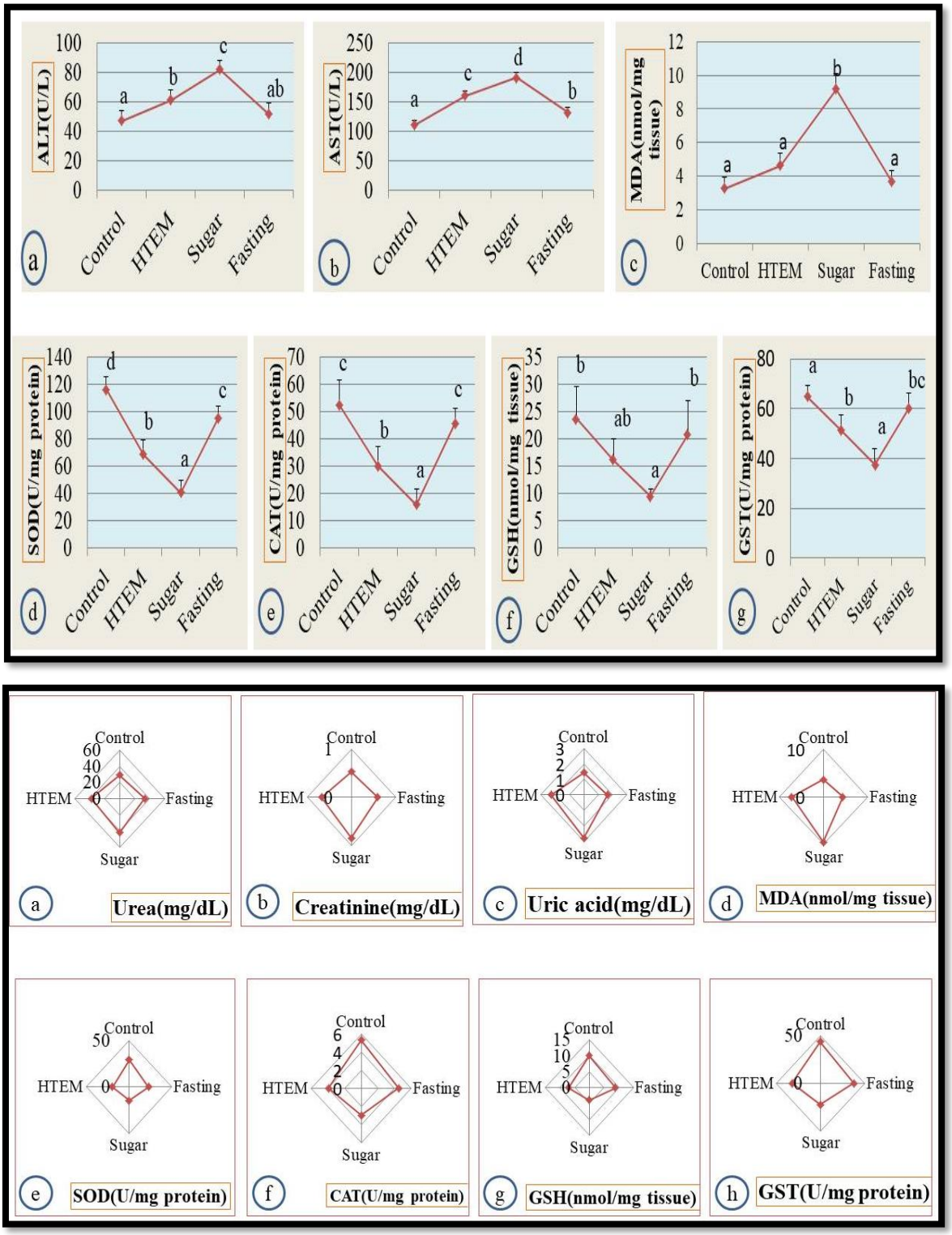
The MDA levels were significantly elevated in the HTEM and Sugar groups compared to the Control ($P < 0.05$). However, fasting reduced MDA compared to the HTEM and Sugar groups.

Antioxidant system

The HTEM and Fasting groups exhibited a significant reduction in CAT, GST, GSH, and SOD expressions compared to the Control group. Conversely, these parameter expressions increased in the Fasting groups compared to the HTEM and Sugar groups (**Figuers 2A–B**).



Figures 1A–B. Values of liver function tests, hepatic oxidative stress biomarkers, and antioxidant system. Data are expressed as means ± SD, n = 5 for each group (Control, HTEM, Sugar, and Fasting); $P < 0.05$ indicates significant differences.



Figures 2A–B. Values of kidney function tests, renal oxidative stress biomarkers, and antioxidant system. Data are expressed as means ± SD, n = 5 for each group (Control, HTEM, Sugar, and Fasting); $P < 0.05$ indicates significant differences.

Histopathological examination of the liver

The liver samples from the Control group exhibited normal histoarchitecture (**Figures 3, a1-a3**). The hepatocytes were closely adjacent and densely organized in anastomosing and branching plates or cords. The hepatocytes exhibited predominantly polyhedral cellular outlines and were distinctly demarcated from one another by capillary-like spaces known as liver sinusoids or blood sinusoids. The central veins exhibited a normal appearance, with a lining of the endothelium layer. The central veins in the HTEM group exhibited clear signs of

enlargement and were present in substantial quantities (**Figures 3, b1–b3**). The expansion of the blood vessels in the liver originated from the HTEM group and extended to the Sugar group (**Figures 3, c1–c3**). The presence of red blood cells (hemorrhage) caused congestion in particular central veins. In addition, the blood sinusoids exhibited mild dilation and occasional congestion in certain regions (Figure 3, b1). The central veins were surrounded by an abundance of mononuclear inflammatory cells, which were rounded in shape. Additionally, there was an observed increase in the number of Kupffer cells. In the Sugar group, the central veins exhibited congestion and mild dilation in certain regions (**Figure 3, c1**). The degraded hepatic parenchyma exhibited focal metastatic cancer cells (bold arrows in **Figures 3, c2–c3**) and inflammatory cell infiltration surrounding the central vein. These cancer cells displayed irregular intima and hepatocyte degeneration. The liver was infiltrated by basophilic cells with a rounded morphology (circles in **Figure 3, c3**). A significant quantity of Kupffer cells was present in the blood sinusoids (squares in **Figure 3, c3**). Vacuolated hepatocytes (arrowheads) with shifted nuclei and cytoplasm towards the periphery were also evident. Additionally, a few hepatocytes may have one or more smaller vacuoles (between brackets in **Figure 3, c3**). In the Fasting group, the liver had a normal appearance, with intact central veins, regular intima (shown by an arrow in **Figure 3, d2**), and well-defined polyhedral hepatocytes.

Fasting reduced high temperature-induced PCNA protein expression in the liver tissues

Figure 4 illustrates the PCNA protein expression in the liver sections of all experimental groups. Liver sections of the Control and Fasting groups showed negative or mild PCNA protein expression in hepatocyte nuclei (**Figures 4, a1–a3 and d1–d3**). Conversely, liver sections of the HTEM (**Figures 4, b1–b3**) and Sugar groups (**Figures 4, c1–c3**) showed acute PCNA protein expression. The PCNA expression in the liver sections was significantly increased in HTEM and Sugar groups compared to the Control and Fasting groups (**Figure 4, e**).

Histopathological examination of murine kidney in all groups

In the control group, the kidney tissue was clearly distinguished into two regions in cross-section: an outer cortex and an inner medulla. The renal corpuscle consists of the glomerulus surrounded by a double-walled epithelial capsule called Bowman's capsule, which appeared normal (bold arrows in **Figures 5, a1–a3**). The proximal convoluted tubules (PCTs) in the control mice have narrow lumens lined by simple cuboidal or columnar epithelium. The distal convoluted tubules (DCTs) were defined with large lumen lined by simple cuboidal epithelial cells with rounded and centrally situated nuclei that also appeared normal (**Figures 5, a1–a3**). In the HTEM group, glomeruli suffered from hypercellularity, leaving no urinary space between the Bowman's capsule and glomerulus (bold arrows in **Figures 5, b1–b3**) called hyperplasia. The proximal tubule cells were characterized by very pale cytoplasm with highly reduced lumen and variable-sized nuclei (**Figures 5, b2–b3**). The distal convoluted tubules differed from their normal architecture with degenerative changes in the form of vacuolated cytoplasm and dilated lumen filled with cellular debris due to the devastation of their epithelium (**Figure 5, b3**). Interstitial tissue was filled with extensive inflammatory cells in between tubules (arrowhead in **Figure 5, b3**) in the form of spindle-shaped inflammatory cells. In the Sugar group, glomeruli exhibited hypercellularity, leaving no urinary space between the Bowman's capsule and glomerulus (**Figures 5, c1–c3**), which is called hyperplasia. In the interstitial tissue, there was obvious interstitial oedema (**Figures 5, c2–c3**). The proximal tubule cells were characterized by very pale cytoplasm, with highly reduced lumen and irregular epithelial cells. The Fasting group showed more or less obvious improvement in the structure of the kidney with normal glomerulus and urinary space (bold arrows and arrows in **Figures 5, d1–d2**) and normal renal tubules (asterisk in **Figure 5, d1**).

WT1 expression in the kidney

The WT1 expression was highly detected in the renal tubules of mice in HTEM and Sugar groups (**Figures 6, b1–b2 and c1–c2**) compared to the Control (**Figures 6, a1–a2**) or Fasting groups (**Figures 6, d1–d2**).

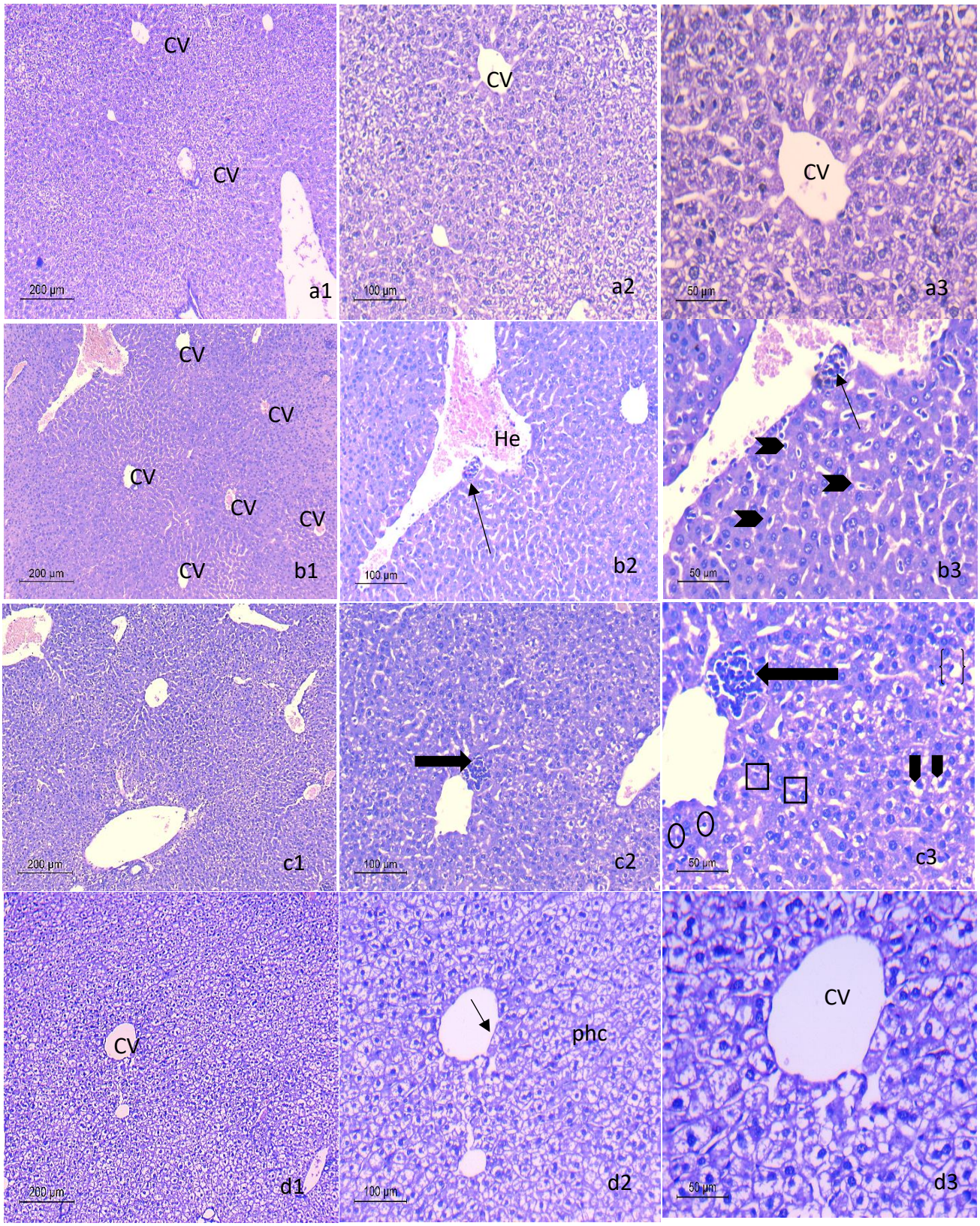


Figure 3. Photomicrographs of liver (**a–d**) sections for the different experimental groups. The H&E-stained liver samples showing (i) normal hepatic architecture in controls (**a1–a3**) within which normal CVs, and normal polyhedral hepatocytes appear; (ii) many CVs appear enlarged, numerous in number and congested with red blood cells in HTEM group (**b1–b3**), focal mononuclear inflammatory cells which are rounded and presence in group (arrow), large number of the Kupffer cells appear (arrowheads); (iii) the CVs are congested and slightly dilated, focal metastatic cancer cells (bold arrows in **c2–c3**) and inflammatory cell infiltration surrounding the CV, CV with irregular intima, rounded basophilic cells invade the liver (circles in **c3**), large number of the Kupffer cells lies in the blood sinusoids (squares in **c3**), vacuolated hepatocytes (arrowheads), nucleus and cytoplasm displaced to the periphery and a few hepatocytes may contain one or more smaller vacuoles are also obvious (between brackets in **c3**) in the Sugar group (**c1–c3**); (iv) normal hepatic architecture, regular CVs intima (arrow) and clear phcs in the Fasting group (**d1–d3**). Abbreviations: CV, central vein; He, hemorrhage; phi, polyhedral hepatocytes.

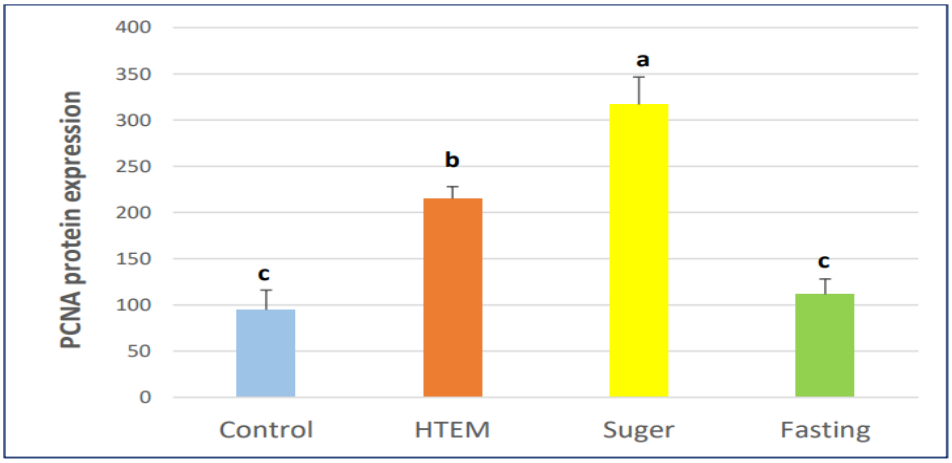


Figure 4. Immunohistochemical staining with PCNA of liver sections (**a–e**) showing the control (**a1–a3**) and Fasting group (**d1–d3**) exhibiting negative or mild PCNA protein expression in hepatocyte nuclei. Conversely, liver sections of the HTEM (**b1–b3**) showed strong PCNA protein expression that was accelerated in the Sugar group (**c1–c3**). PCNA protein expression in the liver sections was significantly increased in Sugar and HTEM mice compared to the Control and Fasting groups (**e**).

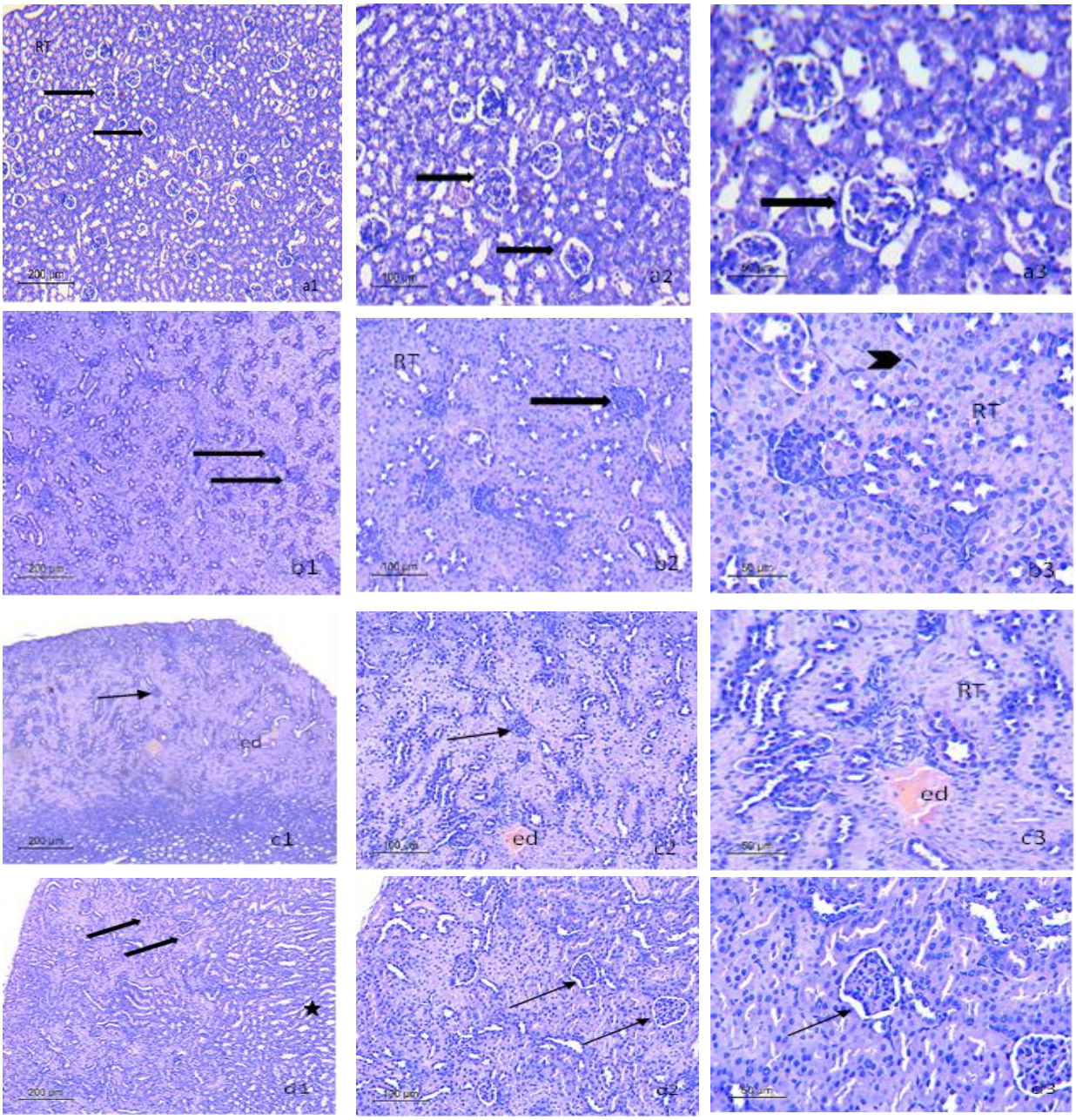


Figure 5. Photomicrographs of kidney sections of different experimental groups (**a–d**). H&E-stained kidney samples showing (i) normal kidney tissue architecture in control (**a1–a3**) with normal glomerulus (bold arrows) and normal RTs; (ii) glomeruli suffered from hyperplasia (bold arrows), very pale cytoplasm of RTs and cellular debris in distal tubules, spindle-shaped inflammatory cells within interstitial tissue (arrowhead) in HTEM group (**b1–b3**); (iii) glomeruli suffered from hyperplasia (arrows), very pale cytoplasm of RTs and interstitial oedema in Sugar group (**c1–c3**); (iv) normal glomerulus with normal urinary space (arrows and bold arrows) and normal RTs (asterisk) in Fasting group (**d1–d3**). Abbreviations: RT, renal tubules; ed, interstitial oedema.

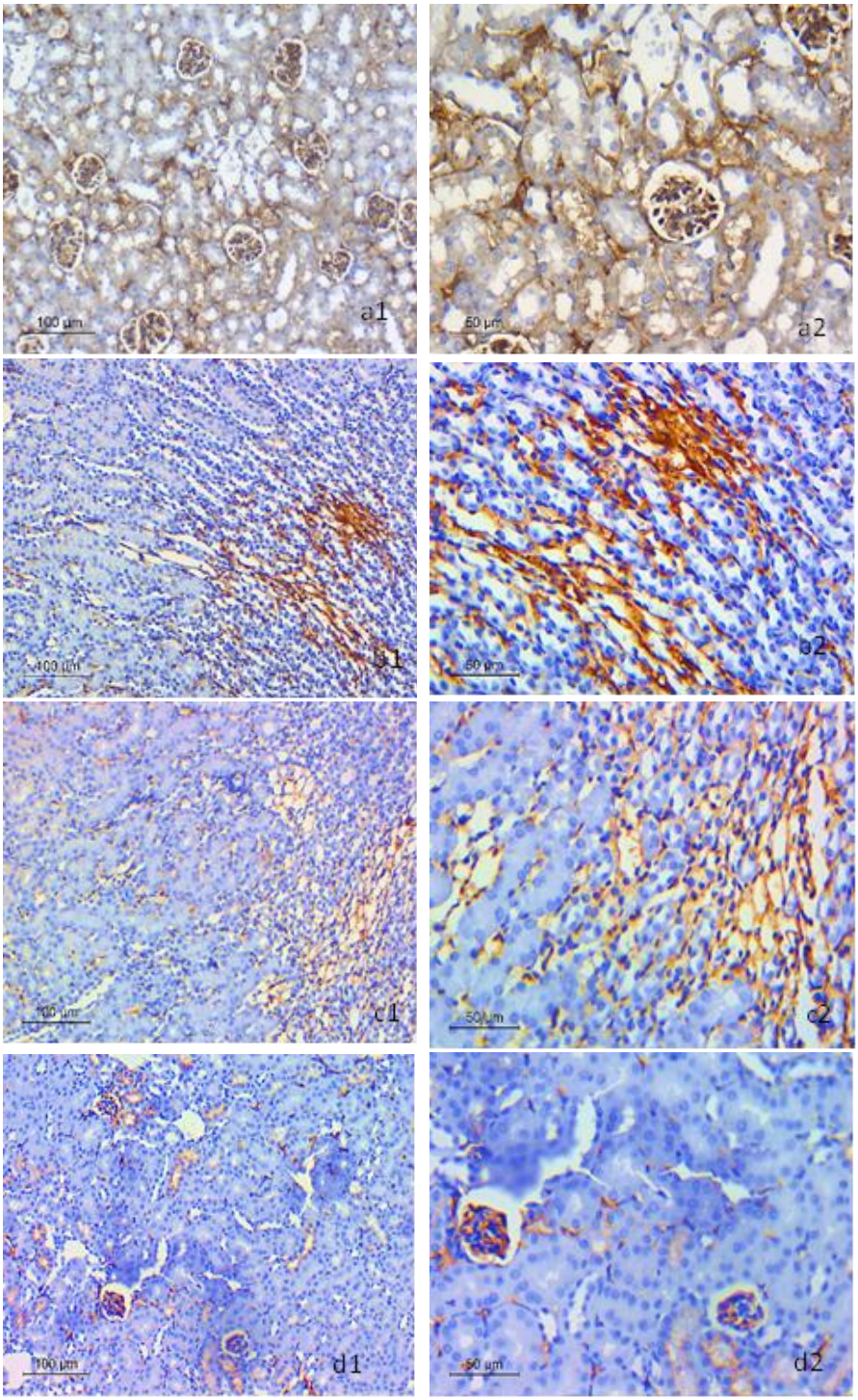


Figure 6. Immunohistochemical staining with WT1 for the kidney sections (**a–d**) shows highly detected WT1 expression in the renal tubules of mice treated with HTEM and Sugar groups (**b1–b2 and c1–c2**) compared to Control or Fasting groups (**a1–a2 and d1–d2**).

Discussion

Liver energy metabolism is tightly controlled; numerous signals originating from diet, hormones, and neurons have been found to affect the metabolism of the amino acids, lipids, and carbohydrates of the liver (32). Glucose is converted into glucose 6-phosphate (G6P) by phosphorylation through the enzyme glucokinase in hepatocytes, reducing glucose levels inside the cells and enhancing glucose absorption even more (33). Herein, in the seventh week, the ALT and AST levels were significantly elevated in the HTEM and Sugar groups compared to the control. Conversely, fasting in mice significantly decreased AST and ALT levels, suggesting that fasting had advantageous outcomes (34). Considering the benefits of fasting in reducing inflammation, regulating glucose levels, and improving liver metabolism, it is advisable to incorporate fasting as a complementary approach to treat steatosis and other hepatic metabolic disorders. The fasting of mice leads to alterations in the biochemical indicators associated with their liver. Consequently, biochemical markers of the physiology or functions of the liver were evaluated (35).

The kidneys are crucial in assisting thermoregulation, cardiovascular control, and water and electrolyte regulation in response to heat stress. A recent study has indicated that heat stress amplifies the susceptibility of the kidneys to pathological occurrences such as acute kidney injury (AKI). Furthermore, engaging in physical activity and experiencing dehydration further intensify this risk. The occurrence of chronic kidney disease (CKD) in work situations is partially attributed to heat stress-induced AKI (36). Maintaining homeostasis relies on the proper functioning of the kidneys; heat stress challenges various physiological processes regulated by the kidneys (37). Our study indicated that the HTEM and Sugar groups exhibited significantly elevated urea, creatinine, and uric acid levels, whereas significantly decreased urea, creatinine, and uric acid levels in the Fasting group.

Our observation revealed that the long-term consumption of glucose-enriched diets elevated lipid profiles, indicating a significant rise in MDA concentration in the HTEM and Sugar groups compared to the control. Additionally, we observed a significant decrease in MDA concentration after fasting in mice from these two groups, consistent with the findings of Ma et al. (17). Prior studies have focused on the correlation between food and its ability to prevent the initiation and dissemination of diseases. A newly developed theorem suggests that meal timing is also important (38). The liver is the main organ responsible for providing the body with energy in a physiological state. Reports have indicated that hepatic glycogen is exhausted within 12–24 hours of fasting. Subsequently, the body transitions into a metabolic state where energy is derived from non-hepatic glucose, ketone bodies formed from fat, and free fatty acids (16). The polyenoic lipid peroxidation occurring in the endoplasmic reticulum produces free radicals that reduce GST levels and the effectiveness of antioxidant enzymes (39), supported by the reduction in CAT, GSH, and GST activities in our study. The enzymes SOD, CAT, GPx, GST, and GSH have been demonstrated to collaborate as a defensive alliance against ROS (7). Consequently, reducing these substances would accumulate superoxide radicals, potentially exacerbating lipid peroxidation (40). The kidney harbors substantial quantities of GST, a cytosolic protein that exhibits strong specificity towards the cells comprising the proximal tubules; urine contains a constant secretion of GST (41). Therefore, oxidative stress, which induces renal vasoconstriction or reduces the glomerular capillary ultrafiltration coefficient, can directly affect renal function (42). This would reduce the glomerular filtration rate, as indicated by the significant decline in kidney function accompanied by the greatly

increased oxidative stress and abnormal evaluation of kidney tissue. Additionally, the elevated urea, uric acid, and creatinine levels in the urine may be attributed to significant leakage due to the hypercellularity of both the glomeruli and tubules (43).

Most studies examining the adverse effects of hyperthermic circumstances have mainly focused on the central nervous system (44). However, there is a scarcity of research on the impact of heat on liver and renal functions, as well as behavioral dysfunctions, particularly in children and infants (45). Here, neonates born to females exposed to hyperthermia exhibited various tissue abnormalities and inflammatory characteristics in both the liver and kidney. The liver is highly susceptible to the effects of high ambient temperature (46). Exposure of environmentally aware animals to heat stress induces an elevation in radical content within the portal venous system, consequently inducing cellular hypoxic stress in the liver (47). A study conducted by Sula et al. has found that when sheep are exposed to heat stress, their kidneys exhibit bilateral enlargement, a pale appearance, and increased humidity (48). Oxidative stress refers to an imbalance between the production of ROS and reactive nitrogen species and the ability of the antioxidant defense system to neutralize them (49,50). This disparity is believed to play a role in the development of multiple neurodegenerative diseases.

Furthermore, heat exposure declines the functioning of antioxidant enzymes and elevates ROS and lipid peroxidation production in various tissues (51). Multiple studies have demonstrated that heat stroke (HS) leads to a significant decrease in the functioning of antioxidant enzymes such as SOD, GPx, and GSH, resulting in mitochondrial dysfunction and excessive production of free radicals. An overabundance of ROS can lead to cellular toxicity and damage to the nervous system, impair the antioxidant defense mechanisms of the body, and increase vulnerability to HS (52-55). The present study observed liver damage in both the HTEM and Sugar groups. Within the HTEM group, the central veins exhibited evident enlargement, congestion, and an increased quantity in certain regions. The liver tissue showed focal infiltration of mononuclear inflammatory cells, which were spherical and had an increased number of Kupffer cells. The results of Roncal-Jimenez et al., who employed mice as experimental subjects (6), corroborate our findings that the livers of mice exposed to heat stress had an elevation in inflammatory macrophages and fibrosis.

A histological examination of the liver of bull calves exposed to heat stress in the jutero revealed a significantly greater number of cells than the livers of bull calves exposed to cooling circumstances in the utero (56). Our findings align with those results and demonstrate an increased number of positive expressions of PCNA in the HTEM and Sugar groups compared to the Control and Fasting groups. Contrary to previous research findings, a study conducted by Chen et al. has shown no observable abnormal liver histology or hepatocyte ultrastructure when exposed to increased heat (46). The HS may be associated with degenerative changes in the renal tubules in animals. Renal failure caused by necrosis and death of the renal tubules typically becomes apparent a few days after experiencing HS (57). The histopathological analysis of renal tissue samples from a study conducted by Miyamoto et al. using a mouse model has revealed the destruction of tubular epithelial cells and the presence of urinary casts (58).

In contrast, the renal tissues of mice subjected to heat stress exhibited signs of proximal tubular injury characterized by brush border loss, mild inflammation, and renal fibrosis (6), consistent with our results. Herein, we observed hypercellularity, very pale cytoplasm, and extensive inflammatory cells in the interstitial tissue between tubules in the HTEM group as well as interstitial edema in the Sugar group. The expression of WT1, a marker of renal tubules, was significantly higher in the HTEM and Sugar groups compared to the Control or Fasting

group. Our study indicated that hyperthermia has detrimental effects on the liver and kidney, as shown in earlier studies of these organs. This study implemented a diet strategy that involved feeding weaning neonates a mixture of food and water with a high sugar content. As a result, the Sugar group experienced significant structural disruption in both the liver and kidney, as observed through morphological and histological analysis. This disruption was more severe than the HTEM and Control groups. These findings can be understood with the research conducted by El Mjiyad et al., which revealed that tumors have a higher rate of glucose absorption and rely on glycolysis instead of respiration, even when oxygen is present (59). Tumor cells that have increased glycolysis show several characteristics, such as accelerated tumor development and a proven capacity to inhibit cell death to varying extents.

Nevertheless, Our study aligns with the findings of Longo and Panda, who have observed that PF and IF offer a range of benefits, including improved disease prevention and the management of existing conditions (11). During the transition from a water medium to a nutrient-rich medium, the absence of glucose and amino acids leads to a shift in the metabolic process. Specifically, the accumulation of acetic acid and ethanol, dependent on glucose and mitochondria, changes to a mode where acetic acid and ethanol are utilized as an energy source instead of glucose, protecting DNA from oxidative damage (60).

Conclusion

Global warming causes irreversible changes to the ecosystem on the Earth. HS, a frequent medical condition related to prolonged exposure to high temperatures, can cause hyperplasia, inflammation, oxidative stress, and multi-organ failure, with the liver and kidneys being particularly vulnerable. Our research has shown that fasting can reduce the stress caused by heat and exacerbated by a sugar-based diet regarding liver and kidney function, which creates a safe prospect of its use in the case of people living under thermal conditions.

Ethics approval

Approval for the animal procedures was granted with approval number AU10230615301.

Consent for publication

All authors listed have approved the manuscript for publication.

Disclosure statement

The authors declared no conflicts of interest.

Availability of data

All data in this study are available from the corresponding author upon request.

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This work received no funding.

Author contributions

E.G. designed the research and wrote the draft of the manuscript; E.H.H. methodology, analyzed the data and edited the manuscript.

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