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Review Article

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Biomarkers of Hyperthermia in Male Wistar Rats: A Comprehensive Review

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ABSTRACT
Hyperthermia, a pathophysiological condition characterized by excessive body heat, triggers systemic, cellular, and molecular stress responses. Male Wistar rats are widely employed as experimental models due to their physiological similarity to humans and reproducible responses to heat stress. Classical biomarkers, including body temperature, corticosterone, peptic ulcers, blood-brain barrier permeability, and fecal pellet output, provide limited insight into the complex molecular cascades induced by heat exposure. This review comprehensively examines biochemical, oxidative stress, inflammatory, renal, hepatic, haematological, and tissue-specific biomarkers in male Wistar rats subjected to hyperthermia. In addition, we explore molecular pathways underlying cellular injury, apoptosis, and organ dysfunction. Understanding these biomarkers facilitates early detection of heat-induced stress, mechanistic studies, and the development of therapeutic interventions.
Keywords: Hyperthermia, Biomarkers, Wistar rats, Cellular injury

INTRODUCTION
Thermoregulation is critical for maintaining homeostasis, and disruption of this balance often leads to hyperthermia when heat production or environmental exposure overwhelms the body's heat-dissipation mechanisms. In experimental models, particularly in male Wistar rats, exposure to temperatures ranging from 38°C–42°C induces a wide spectrum of physiological, biochemical, and cellular changes that mimic human heat-related illnesses (Ippolito *et al*. 2014). Traditionally, biomarkers such as body temperature, corticosterone levels, peptic ulcers, and fecal pellet output have been monitored as primary indicators of stress (Endo *et al*. 2001) (Murphy *et al*. 1979) (Barone et al., 1990). However, recent studies have demonstrated that these classical parameters provide only a limited view of the complex molecular and cellular cascades associated with hyperthermia and may fail to fully capture organ-specific injury, systemic inflammation, or the long-term consequences of heat stress (Yin *et al*. 2025; Jin *et al*. 2025). For instance, a 2024 study observed significant changes in the number and morphology of leukocytes in Wistar rats exposed to 41°C and 44°C, highlighting the importance of immune system monitoring in hyperthermic conditions (Ajanovic *et al*. 2024). Additionally, research by Bijani et al. (2024) demonstrated that scrotal hyperthermia at 43°C induced oxidative stress, apoptosis, and inflammation in testicular tissue, underscoring the need for molecular-level assessments to understand heat-induced organ damage (Bijlani *et al*. 2024). These findings suggest that researchers are increasingly investigating additional biomarkers that provide a more holistic and mechanistic understanding of the hyperthermic response.

Several investigations reported similar changes in the expression of heat shock proteins (HSPs), markers of oxidative stress, and inflammatory cytokines in hyperthermia-exposed rats (Kang *et al*. 2024). These non-classical biomarkers are now recognized as highly sensitive indicators of cellular defense responses and systemic damage (Xu *et al*. 2024). For example, HSP70 and HSP90 are consistently upregulated in vital organs, reflecting an immediate protective response against protein denaturation and aggregation (Zhang and Qi 2025). Oxidative stress markers such as malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH) levels provide reliable insight

into mitochondrial dysfunction and reactive oxygen species (ROS)–mediated injury (Ding and Gao 2025). Likewise, pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, are well-established hallmarks of the hyperthermia-induced inflammatory cascade (Baindara *et al*. 2025). Emerging data indicate that hyperthermia not only triggers these systemic stress markers but also disrupts organ-specific functions. Renal biomarkers such as creatinine and blood urea nitrogen (BUN) reflect acute kidney stress (Thammitiyagodage *et al*. 2020), while hepatic enzymes such as ALT, AST, and ALP reveal hepatocellular injury (Zhang *et al*. 2018). Hematological alterations, including hemoconcentration, leukocytosis, and coagulation imbalances, further demonstrate the systemic nature of hyperthermia (Iba *et al*. 2025). Confirmatory evidence comes from histopathological observations of hepatocyte degeneration, renal tubular necrosis, and cardiac fibre injury, as well as apoptotic markers such as caspase-3 and the Bax/Bcl-2 ratio, which establish that hyperthermia can lead to both reversible stress responses and irreversible cell death (Dervisevic *et al*. 2023).

Despite the growing body of evidence, conflicting findings exist regarding the temporal sequence and severity of these responses. Some authors reported that oxidative stress precedes the inflammatory cascade, suggesting that ROS may act as the primary driver of downstream signalling (Ding and Gao 2025). Others have observed simultaneous activation of oxidative and inflammatory pathways, indicating that both processes may occur in parallel (Cantet *et al*. 2021). Variability in exposure protocols, such as differences in temperature intensity, duration of heat stress, recovery phases, and animal strain, also complicates the interpretation and comparison of results across different studies. This inconsistency highlights the urgent need for standardized experimental approaches and comparative analyses to establish reproducible biomarkers of hyperthermic stress (Palasz *et al*. 2025).

Overall, a number of authors suggested that understanding hyperthermia requires integration of biochemical, oxidative, inflammatory, renal, hepatic, haematological, histopathological, and apoptotic biomarkers, rather than relying solely on traditional indicators such as corticosterone or body temperature. Such a multidimensional perspective not only allows for a comprehensive evaluation of hyperthermic stress but also provides insight into organ-specific vulnerability, cellular stress pathways, and therapeutic targets. Therefore, this review consolidates the available evidence on these biomarkers, their measurement techniques, and the molecular mechanisms underlying hyperthermia-induced damage in male Wistar rats. By synthesizing findings from scattered literature, it aims to create a unified framework that will help researchers assess the severity of heat stress, interpret experimental outcomes, and design effective strategies for future investigations.

BIOCHEMICAL BIOMARKERS

Heat Shock Proteins (HSPs): Molecular Chaperones in Cellular Stress Responses

Heat shock proteins (HSPs) are highly conserved molecular chaperones that play a critical role in cellular defense mechanisms by protecting proteins from stress-induced denaturation and maintaining protein homeostasis. Among the various HSP families, HSP70 and HSP90 have been the most extensively studied in hyperthermia and thermal stress models due to their robust inducibility and functional significance.

Under conditions of elevated temperature, oxidative stress, or other physiological insults, the expression of HSPs increases significantly in vital organs such as the liver, kidney, heart, and brain. This upregulation is closely associated with both the duration and intensity of heat exposure, indicating a dose-dependent protective response (Masud *et al*. 2025). For instance, studies have demonstrated that heat exposure at 42°C induced enhanced expression of HSP70 and HSP90 proteins in cardiomyocytes, leading to the release of Smac from the mitochondria, which is associated with apoptosis regulation (Ding and Gao 2025).

Mechanisms of Action

Functionally, HSPs facilitate the refolding of denatured proteins, prevent the formation of toxic protein aggregates, and interact with components of apoptotic signaling pathways to promote cell survival. The underlying mechanism involves the activation of heat shock factor 1 (HSF1), a transcription factor that, upon sensing cellular stress, undergoes trimerization and translocates into the nucleus (Bardelcik *et al*. 2025). HSF1 binds specifically to heat shock elements (HSEs) present in the promoter regions of HSP genes, thereby initiating transcription and leading to rapid synthesis of HSPs. Recent studies have highlighted that HSF1 serves as a molecular crowding sensor, trimerizing to initiate protective responses that enhance chaperone activities to restore homeostasis (Simoncik *et al*. 2024).

Diagnostic and Experimental Assessment

Quantitative and qualitative assessment of HSP expression is typically carried out using techniques such as Western blotting, enzyme-linked immunosorbent assay (ELISA), and immunohistochemistry, which allow for precise evaluation of their spatial and temporal dynamics in different tissues. In cell and tissue extracts, ELISA is a prominent method used to quantify heat shock proteins, including HSP90α (Makhoba 2025). Additionally, Western blotting has been employed to measure the expression levels of apoptotic and PI3K/AKT/mTOR pathway proteins, providing insights into the regulatory networks involving HSPs (Liu *et al*. 2025 (a)).

Translational Implications

Collectively, the study of HSPs provides important insights into the cellular protective mechanisms against hyperthermia and offers potential translational applications in monitoring heat-induced tissue damage. For example, HSP70 expression has been linked to the

resolution of inflammation, highlighting its role in modulating immune responses during stress conditions (Schroeder *et al*. 2024). Furthermore, the modulation of HSF1 activity through the development of small molecules emerges as a promising therapeutic strategy for disease treatment, as dysfunction of HSF1 contributes to the pathogenesis of various diseases, including cancer (Zhang and Tan 2025).

Lactate

Under conditions of hyperthermia, the metabolic demands of tissues often increase substantially, sometimes surpassing the available oxygen supply (Squier *et al*. 2024). This imbalance between oxygen demand and delivery can force cells to shift from aerobic to anaerobic metabolism, leading to enhanced glycolytic activity and the accumulation of lactate (Kanamori *et al*. 2021). Elevated lactate levels serve as a hallmark of metabolic stress and are indicative of tissue hypoxia and impaired energy homeostasis (Liu *et al*. 2025 (b)) (Yao *et al*. 2024). Persistent lactate accumulation can disrupt cellular pH balance, impair enzyme function, and compromise overall tissue integrity, thereby exacerbating the detrimental effects of heat stress (Falter *et al*. 2024). The measurement of lactate concentration provides valuable insights into the extent of metabolic derangement induced by hyperthermia and can serve as a sensitive biomarker of cellular stress (Libre *et al*. 2025). Various techniques are employed for lactate assessment, including spectrophotometric lactate assays, which allow precise quantification in tissue or plasma samples, and portable lactate analyzers, which offer rapid and convenient point-of-care evaluation. Monitoring lactate dynamics under heat stress conditions not only enhances our understanding of the metabolic consequences of hyperthermia but also aids in evaluating the efficacy of protective interventions and adaptive responses in affected tissues.

Oxidative Stress Markers

Hyperthermia is well known to enhance the production of reactive oxygen species (ROS), leading to a state of oxidative stress that can cause extensive damage to cellular macromolecules, including lipids, proteins, and nucleic acids (Belhadj *et al*. 2014). The excessive generation of ROS during heat stress overwhelms the endogenous antioxidant defense systems, resulting in oxidative modifications that compromise cellular structure and function (Aryal *et al*. 2025; Singh *et al*. 2025). Key biomarkers of oxidative stress in hyperthermic conditions include malondialdehyde (MDA), a product of lipid peroxidation that serves as a reliable indicator of membrane damage; superoxide dismutase (SOD), an enzymatic antioxidant that catalyzes the dismutation of superoxide radicals into hydrogen peroxide; catalase, which further detoxifies hydrogen peroxide into water and oxygen, thereby mitigating ROS-mediated damage; and glutathione (GSH), a critical non-enzymatic antioxidant, whose reduced levels reflect the depletion of cellular antioxidant capacity and heightened vulnerability to oxidative injury (Carmo *et al*. 2022). Mechanistically, hyperthermia promotes mitochondrial ROS generation, disrupts electron transport chains, and depletes cellular antioxidant reserves, culminating in oxidative damage that contributes to organ dysfunction and impaired physiological homeostasis (Zhao *et al*. 2019). The assessment of oxidative stress markers is accomplished through various methodologies, including thiobarbituric acid reactive substances (TBARS) assay for MDA, spectrophotometric assays for enzymatic activities, enzyme-linked immunosorbent assays (ELISA), and high-performance liquid chromatography (HPLC), all of which allow precise evaluation of the oxidative status in tissues and biofluids (Ruiz-Ojeda *et al*. 2018). Monitoring ROS and antioxidant dynamics under hyperthermic conditions provides critical insights into the pathophysiological consequences of heat stress and the efficacy of potential therapeutic interventions aimed at mitigating oxidative damage (Hong *et al*. 2024).

Glucose and Electrolyte Disturbances

Hyperthermia profoundly affects systemic metabolic homeostasis, often resulting in disturbances in glucose levels and electrolytes. Exposure to elevated temperatures activates the sympathetic-adrenal-medullary (SAM) axis, leading to increased secretion of catecholamines such as adrenaline and noradrenaline (Huang *et al*. 2024) (Afsal *et al*. 2018). This hormonal surge promotes gluconeogenesis and glycogenolysis, resulting in hyperglycemia, which reflects heightened metabolic activity and stress. Concurrently, electrolyte imbalances may occur, with fluctuations in sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) levels arising from heat-induced dehydration, increased renal excretion, and impaired electrolyte reabsorption (Liu *et al*. 2025) (c). These disturbances not only compromise cellular and organ function but also serve as early systemic biomarkers of heat-induced stress, providing insight into the physiological adaptations and potential risks associated with hyperthermia. Accurate measurement of these parameters is achieved using biochemistry analyzers for glucose, ion-selective electrodes for precise ion quantification, and flame photometry for assessing sodium and potassium levels (Oliviera *et al*. 2017). Continuous monitoring of glucose and electrolyte dynamics under hyperthermic conditions is crucial for understanding the systemic impact of heat stress and for developing strategies to mitigate its deleterious effects on organ function and overall homeostasis (Burhans *et al*. 2022).

Inflammatory Biomarkers

Heat exposure triggers systemic inflammation through activation of immune and stress pathways.

Cytokines

Hyperthermia induces a pronounced inflammatory response characterized by the elevation of pro-inflammatory cytokines, which serve as important biomarkers of cellular stress and tissue injury (Heled *et al*. 2013). Key cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and

interleukin-6 (IL-6), have been consistently reported to increase in both serum and various tissues following exposure to elevated temperatures (Cantet *et al*. 2021; Heled *et al*. 2013). The upregulation of these cytokines reflects the activation of intracellular signaling pathways, particularly the nuclear factor kappa B (NF-κB) pathway, which orchestrates the transcription of multiple inflammatory mediators in response to cellular stress (Chen *et al*. 2024). Elevated cytokine levels not only indicate the presence of an acute-phase inflammatory response but also contribute to systemic effects such as fever, vascular permeability changes, and recruitment of immune cells to affected tissues (Leon *et al*. 2002). Measurement of these inflammatory biomarkers is commonly performed using enzyme-linked immunosorbent assays (ELISA), multiplex cytokine assays, and immunohistochemical techniques, enabling precise quantification and localization of cytokine expression (Leng *et al*. 2008). Monitoring cytokine dynamics during hyperthermia provides critical insights into the interplay between thermal stress, immune activation, and tissue injury, highlighting their potential as both diagnostic and prognostic markers in heat-induced pathophysiology (Cantet *et al*. 2021).

Acute-Phase Proteins

C-reactive protein (CRP) is a key acute-phase protein whose levels rise rapidly in response to systemic inflammation, making it a valuable biomarker for heat-induced stress and tissue injury (Mirsanei *et al*. 2024). During hyperthermia, the excessive generation of reactive oxygen species (ROS) and subsequent cellular damage serve as potent stimuli for the activation of intracellular signalling pathways, particularly the nuclear factor kappa B (NF-κB) pathway (Wang *et al*. 2025). Activation of NF-κB promotes the transcription of multiple inflammatory mediators, including CRP and pro-inflammatory cytokines, thereby amplifying the systemic inflammatory response (Agrawal *et al*. 2003). Elevated CRP levels reflect both the presence and intensity of this acute-phase response, providing insight into the degree of physiological stress experienced by the organism. Quantitative assessment of CRP can be performed using enzyme-linked immunosorbent assays (ELISA), while molecular approaches such as quantitative PCR allow evaluation of cytokine mRNA levels associated with its induction (Ansar and Ghosh 2016). Immunohistochemistry further enables the localization and semi-quantitative analysis of CRP expression in tissues (Di *et al*. 2012). Monitoring CRP dynamics in conjunction with other inflammatory biomarkers not only facilitates the assessment of systemic inflammation under hyperthermic conditions but also aids in understanding the mechanistic links between oxidative stress, cellular damage, and immune activation (Dobos *et al*. 2024).

Renal and Hepatic Biomarkers

Renal Biomarkers

Hyperthermia exerts significant effects on renal physiology, often leading to acute renal stress and functional impairment (Goto *et al*. 2023). Exposure to elevated temperatures can cause renal ischemia, tubular injury, and decreased glomerular filtration, thereby compromising the kidney's ability to maintain fluid and electrolyte balance (Amorim and Schlader 2025). Key biomarkers for assessing renal function under heat stress include serum creatinine and urea, whose elevated levels serve as direct indicators of impaired filtration and renal dysfunction (Chapman *et al*. 2021). Additionally, the blood urea nitrogen (BUN) to creatinine ratio provides valuable information on hydration status and renal perfusion, helping to distinguish between prerenal and intrinsic renal injury (Uchino *et al*. 2012). Accurate measurement of these renal biomarkers is commonly achieved using automated biochemistry analyzers, which allow rapid and precise quantification, or through colorimetric assay kits that provide a cost-effective alternative for laboratory evaluation (Tarim and Tekin 2024). Monitoring these parameters under hyperthermic conditions offers critical insight into the degree of renal compromise, enabling early detection of heat-induced nephrotoxicity and guiding interventions aimed at preserving renal function (Butler-Dawson *et al*. 2024).

Hepatic Biomarkers

The liver, as a central metabolic and detoxifying organ, is highly susceptible to damage under hyperthermic conditions (Balibar *et al*. 2024). Elevated body temperatures can induce hepatocellular stress, leading to oxidative damage, inflammatory cell infiltration, and apoptosis of hepatocytes, thereby compromising liver function (St *et al*. 2022). Key biomarkers for assessing hepatic injury include aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), whose elevated levels in serum are indicative of hepatocellular damage and cholestatic disturbances (Gupta 2024). Additionally, hyperbilirubinemia, reflected by increased serum bilirubin levels, serves as a marker of impaired hepatic metabolic capacity and bile excretion (Rochling 2021). These biomarkers collectively provide critical information regarding the extent and severity of liver injury induced by hyperthermia (Wang *et al*. 2022). The measurement of hepatic enzymes and bilirubin is routinely performed using automated biochemistry analyzers, allowing rapid, accurate, and reproducible assessment (Romijn *et al*. 2023). Monitoring hepatic biomarkers under heat stress conditions is essential for evaluating organ-specific injury, understanding the pathophysiological consequences of thermal stress, and developing strategies to mitigate liver dysfunction during hyperthermic episodes (Gao *et al*. 2024).

Hematological Biomarkers

Hyperthermia exerts profound effects on the hematological system, leading to systemic alterations that reflect dehydration, immune activation, and changes in coagulation status (Borgman *et al*. 2019). Elevated body temperature often results in hemoconcentration, which is manifested as increased hematocrit and hemoglobin levels, indicating reduced plasma volume

and fluid loss (Komka *et al*. 2022). In addition, the leukocyte profile is frequently altered under heat stress, with leukocytosis or lymphopenia reflecting immune activation and the body's attempt to respond to cellular stress and potential tissue injury (Llanos-Garrido *et al*. 2023). Platelets and coagulation markers are also affected, as hyperthermia can enhance coagulability, increasing the risk of thrombotic events, or in some cases induce mild thrombocytopenia, highlighting the complex impact of thermal stress on hemostasis (Ke *et al*. 2024). Assessment of these hematological parameters is typically performed using automated hematology analyzers for rapid and precise evaluation, supplemented by manual counts and specialized coagulation assays to examine platelet function and clotting potential (Anyiam *et al*. 2024). Monitoring hematological biomarkers under hyperthermic conditions provides critical insights into the systemic physiological responses to heat stress and aids in understanding the integrated effects on immune function, fluid balance, and hemostatic integrity (Llanos-Garrido *et al*. 2023).

Tissue-Specific and Cellular Biomarkers

Histopathology

Histopathological analysis provides critical insights into the structural and cellular consequences of hyperthermia on vital organs. In the liver, heat stress often induces hepatocyte necrosis, cytoplasmic vacuolization, and sinusoidal congestion, reflecting direct cellular injury and impaired metabolic function (Shokry *et al*. 2024). The kidneys exhibit tubular degeneration, interstitial edema, and occasional glomerular changes, indicative of compromised filtration and renal ischemia resulting from thermal stress (Rebez *et al*. 2023) (Ding *et al*. 2011). Cardiac tissues are also affected, with myocardial fiber injury, cytoplasmic vacuolization, and occasional inflammatory infiltration, highlighting the vulnerability of the heart to elevated body temperatures (Llanos-Garrido *et al*. 2023). These organ-specific histopathological alterations are typically assessed using hematoxylin and eosin (H&E) staining for general tissue architecture, light microscopy for detailed morphological evaluation, and electron microscopy for ultrastructural examination of subcellular damage. Incorporating histopathological observations alongside biochemical, molecular, and hematological biomarkers provides a comprehensive understanding of the pathophysiological impact of hyperthermia and offers valuable evidence for evaluating tissue-specific injury and the effectiveness of potential protective interventions (Rebez *et al*. 2023). Organ-specific effects of hyperthermia, along with the corresponding biomarkers involved, are summarized in Fig. 1.

Apoptosis Markers

Hyperthermia is a potent inducer of apoptosis, activating both intrinsic (mitochondrial) and extrinsic cell death pathways, and contributing to tissue injury under thermal stress conditions (Lukácsi *et al*. 2024). One of the key hallmarks of apoptosis in hyperthermic models is the activation of caspase-3, an executioner caspase that orchestrates the cleavage of cellular substrates leading to programmed cell death. In addition, the balance between pro-apoptotic and anti-apoptotic members of the Bcl-2 family, particularly the Bax/Bcl-2 ratio, serves as a critical indicator of cellular susceptibility to apoptosis, with a shift toward Bax signaling reflecting enhanced pro-apoptotic activity (Wang *et al*. 2019) (Wu *et al*. 2023). Mechanistically, hyperthermia-induced reactive oxygen species (ROS) generation, intracellular calcium dysregulation, and mitochondrial membrane depolarization converge to initiate apoptotic cascades, ultimately leading to cell death and tissue dysfunction (Tanaka *et al*. 2025).

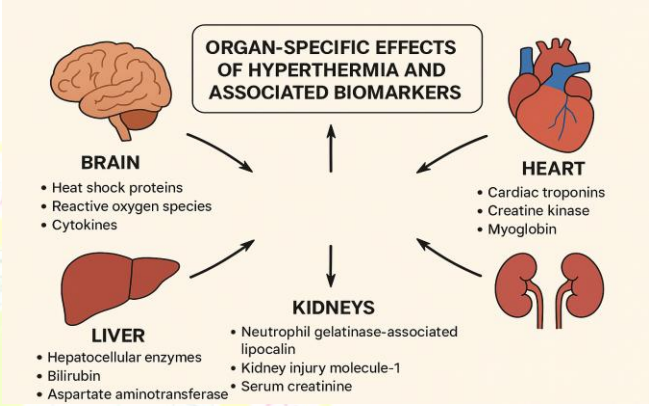

Figure 1. Organ-specific effects of hyperthermia and associated biomarkers.

The assessment of apoptotic markers is commonly performed using Western blotting to detect protein expression (Hirano *et al*. 2012), immunohistochemistry for tissue localization (Bressenot *et al*. 2009), and flow cytometry for quantitative evaluation of apoptotic cell populations (Alshehade *et al*. 2024). Monitoring apoptosis under hyperthermic conditions provides essential insights into the molecular mechanisms of heat-induced tissue injury and aids in understanding the interplay between oxidative stress, mitochondrial dysfunction, and programmed cell death in organ-specific pathology (Marquez-Acevedo *et al*. 2023).

Organ-Specific Molecular Pathways

Oxidative Stress Pathway

Hyperthermia triggers a cascade of molecular events that culminate in oxidative stress and organ-specific cellular injury. Elevated body temperatures enhance the generation of reactive oxygen species (ROS), which initiate lipid peroxidation, as indicated by increased malondialdehyde (MDA) levels, and induce oxidative damage to proteins and nucleic acids (Belhadj Slimen *et al*. 2014). This accumulation of oxidative damage disrupts mitochondrial integrity, impairing energy production and leading to mitochondrial dysfunction. Mitochondrial damage, in turn, facilitates the release of pro-apoptotic factors such as cytochrome c, activating caspase cascades and ultimately promoting apoptosis (Wang *et al*. 2013). The oxidative stress pathway is therefore a central mechanism linking hyperthermia-induced molecular perturbations to cellular and tissue injury across multiple organs. Monitoring markers such

as ROS, MDA, and mitochondrial integrity, alongside apoptotic indicators like caspase-3 activation and Bax/Bcl-2 ratio, provides a comprehensive understanding of the molecular underpinnings of hyperthermia-induced organ damage (BayIr *et al*. 2008).

Inflammatory Pathway

Hyperthermia induces a robust inflammatory response through the activation of key intracellular signaling cascades, primarily the nuclear factor kappa B (NF-κB) pathway (Selkirk *et al*. 2008). Elevated temperatures and heat-induced cellular damage stimulate NF-κB translocation into the nucleus, where it promotes transcription of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) (Fukano *et al*. 2008). The resultant cytokine surge triggers systemic inflammation, contributing to vascular alterations, immune cell recruitment, and tissue injury across multiple organs. C-reactive protein (CRP), an acute-phase protein, is also upregulated downstream of NF-κB activation, serving as an additional systemic marker of inflammation (Ridker *et al*. 2016). Together, these molecular events link cellular stress to organ dysfunction, highlighting the central role of the inflammatory pathway in the pathophysiology of hyperthermia. Measurement of these markers using ELISA, quantitative PCR, and immunohistochemistry provides insight into both the intensity and temporal dynamics of heat-induced inflammatory responses (Hashim *et al*. 2010).

Apoptotic Pathway

Hyperthermia induces apoptosis through both intrinsic (mitochondrial) and extrinsic pathways, with the intrinsic pathway being particularly prominent in heat-stressed tissuesElevated reactive oxygen species (ROS) and mitochondrial dysfunction trigger an imbalance in Bcl-2 family proteins, characterized by an increase in pro-apoptotic Bax and a decrease in anti-apoptotic Bcl-2. This shift promotes mitochondrial outer membrane permeabilization and the release of cytochrome c, which activates initiator caspase-9. Subsequently, the executioner caspase-3 is activated, leading to the cleavage of cellular substrates and the morphological and biochemical hallmarks of programmed cell death. This apoptotic cascade under hyperthermia contributes significantly to tissue injury in vital organs, including the liver, kidney, and heart. Detection of apoptotic events is commonly performed using Western blotting for caspases, immunohistochemistry for protein localization, and flow cytometry for quantifying apoptotic cell populations, providing a comprehensive view of heat-induced cell death mechanisms (Gorbaslieva et al., 2025). The interrelated molecular pathways linking oxidative stress, inflammation, and apoptosis under hyperthermic conditions are depicted in Fig. 2.

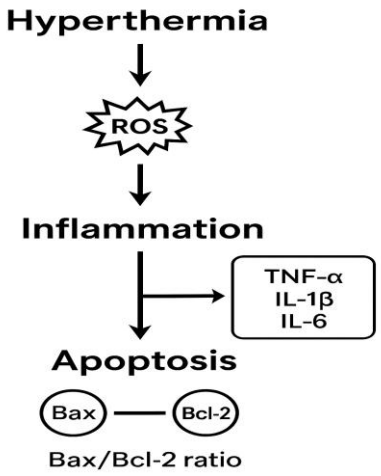

Figure 2. Molecular pathways of oxidative stress, inflammation, and apoptosis in hyperthermia.

Table 1. Summary Table of Key Biomarkers

Biomarker	Tissue/Source	Measurement Method	Mechanism / Significance	Reference
HSP70, HSP90	Liver, Kidney, Heart	Western blot, ELISA, IHC	Molecular chaperone; protects against protein denaturation	Yamamoto et al., 2018
Lactate	Blood	Spectrophotometry	Anaerobic metabolism; indicates tissue hypoxia	Leite et al., 2020
MDA	Liver, Kidney	TBARS assay	Lipid peroxidation	Leite et al., 2020
SOD, Catalase, GSH	Liver, Heart, Brain	Spectrophotometry, ELISA	Antioxidant defense	Leite et al., 2020
TNF-α, IL-1β, IL-6	Serum, Liver	ELISA, qPCR	Inflammatory response	Horowitz, 2014
CRP	Serum	ELISA	Acute-phase inflammatory marker	Horowitz, 2014
Creatinine, Urea	Blood	Biochemistry analyzer	Renal function marker	Leite et al., 2020
AST, ALT, ALP	Blood	Biochemistry analyzer	Hepatic injury	Yamamoto et al., 2018
Hematocrit, Hemoglobin	Blood	Hematology analyzer	Dehydration, hemoconcentration	Morton & Griffiths, 2012
Leukocyte count	Blood	Hematology analyzer	Immune stress	Morton & Griffi

Biomarker	Tissue/Source	Measurement Method	Mechanism / Significance	Reference
				ths, 2012
Caspase-3, Bax/Bcl-2	Liver, Kidney	Western blot, IHC	Apoptosis	Leite et al., 2020
Histopathology	Liver, Kidney, Heart	H&E staining, microscopy	Tissue injury	Yamamoto et al., 2018

oxidative stress, inflammation, and apoptosis under hyperthermic conditions are depicted in Fig. 2.

Table 2. Summary of recent studies exploring oxidative stress, inflammation, and histopathological alterations in hyperthermia-exposed male rats. The table highlights each study's year, key findings, and relevance to biomarkers, organ-specific damage, and potential therapeutic approaches relevant to the current research on heat-induced physiological stress.

Study	Key Findings & Relevance
Effect of prolonged whole-body hyperthermia on adult male rat testes and the protective role of vitamin C and E (2022) PubMed	Found that 1 hr/day at 41°C for 2 weeks causes oxidative stress (↑MDA, ↓SOD, catalase, GPx, GSH) and decreased testosterone, testicular damage in Wistar rats. Very relevant for your oxidative stress + biochemical biomarkers sections. PubMed
Heat Stress Alters Oxidative and Inflammatory Responses in Many Tissues of Male Rats (2023) bezmialemscience.org	Exposed rats at 30°C and 35°C; found changes in IL-2, IL-6, TNF-α, MPO, SOD in brain, testis, heart, liver etc. Good for your inflammatory & oxidative stress sections. bezmialemscience.org
Camel Whey Protein Attenuates Acute Heat Stress-Induced Kidney Injury in Rats (2024) MDPI	Shows that a treatment (camel whey protein) modulates oxidative stress via PI3K/AKT/eNOS in kidney injury caused by heat stress. Useful for organ-specific biomarkers & therapeutic angle. MDPI
Protective impact of Betanin against noise and scrotal hyperthermia on testicular toxicity in Wistar rat (2024) PubMed	Combines hyperthermia + noise stress; measures MDA, GSH, TNF-α, IL-6, apoptosis markers. Useful for apoptosis + oxidative + inflammatory triangulation. PubMed

DISCUSSION

The systemic response to hyperthermia in male Wistar rats is highly complex and involves a cascade of interconnected biochemical, physiological, and cellular processes (Freedman 2002). Hyperthermia disrupts homeostasis at multiple levels, leading to a chain reaction that begins with metabolic and molecular alterations and eventually manifests as organ-specific injury (Ognjanovic *et al*. 2002). A comprehensive understanding of these processes is crucial, as it provides insights into the mechanisms of heat-induced morbidity and mortality and also aids in the identification of potential biomarkers and therapeutic targets (Khan and Brown 2002). At the molecular level, biochemical markers such as heat shock proteins (HSPs) and lactate serve as early indicators of stress (Mel'nikov *et al*. 2000). HSPs are well-known molecular chaperones that become upregulated in response to elevated temperature, acting as a protective mechanism to preserve protein stability and prevent aggregation (Bønløkke *et al*. 1999). Their induction reflects the activation of cellular defense pathways. Similarly, elevated lactate levels represent a shift in energy metabolism due to impaired mitochondrial oxidative phosphorylation and increased reliance on anaerobic glycolysis, signifying metabolic imbalance under heat stress conditions (Osorio *et al*. 2003). Oxidative stress markers provide further evidence of cellular dysfunction. Hyperthermia enhances the generation of reactive oxygen species (ROS), which in excess can overwhelm antioxidant defenses such as superoxide dismutase (SOD), catalase, and glutathione (Hong *et al*. 2014). This imbalance leads to oxidative damage of lipids, proteins, and nucleic acids. Such damage not only impairs cell function but also acts as a trigger for downstream signaling cascades (Randall *et al*. 2013). ROS are known to activate transcription factors such as NF-κB, which in turn upregulate pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6, thus linking oxidative stress with inflammation (Belhadj Slimen *et al*. 2014). The inflammatory response is another hallmark of hyperthermia. Elevated cytokine levels signify immune system activation and the initiation of systemic inflammatory response syndrome (SIRS)-like conditions (Guillermo *et al*. 2015). This can exacerbate tissue damage through leukocyte infiltration, release of proteases, and additional ROS production, ultimately creating a vicious cycle of injury (Ganesan *et al*. 2016).

Comparative analysis of acute versus chronic hyperthermia

The physiological and molecular responses to hyperthermia vary significantly between acute and chronic exposures. Acute hyperthermia, typically induced by short-term exposure (1–4 hours) at higher temperatures (≥42°C), elicits rapid activation of stress-response pathways, including upregulation of heat shock proteins (HSP70, HSP90), oxidative stress generation, and transient inflammatory responses (Singh *et al*. 2024). These effects are often reversible upon recovery

if thermal damage remains below necrotic thresholds. In contrast, chronic hyperthermia, involving prolonged or repeated exposure to moderately elevated temperatures (38–40°C) over several days or weeks, leads to cumulative physiological stress, persistent ROS production, and sustained inflammatory signaling (Yang *et al*. 2025). This chronic insult can result in mitochondrial dysfunction, cellular apoptosis, and progressive organ damage, particularly in metabolically active tissues such as the liver, kidney, and brain. The comparison highlights that temperature intensity and duration are critical determinants of cellular outcome acute hyperthermia primarily triggers protective responses, while chronic exposure often culminates in irreversible injury and organ dysfunction (Ding and Gao 2025).

Novelty and integrative scope of this review

A key strength of the present review lies in its comprehensive integration of biochemical, histopathological, and apoptotic biomarkers in hyperthermic male Wistar rats. While previous reviews have often focused on individual organ systems or isolated biomarker categories, this work unites findings across multiple physiological domains—oxidative stress, inflammation, apoptosis, and tissue pathology to construct a holistic framework of systemic heat-induced injury. By correlating biochemical markers (e.g., MDA, SOD, GSH), inflammatory mediators (e.g., TNF-α, IL-6, CRP), and apoptotic indicators (e.g., caspase-3, Bax/Bcl-2 ratio) with organ-specific histopathological findings, the review establishes an integrated understanding rarely addressed in the existing literature. This multidimensional synthesis enhances the translational relevance of animal models for evaluating the progression and severity of hyperthermic stress.

Translational relevance

These findings closely parallel the pathophysiological mechanisms observed in human heat stroke, where oxidative stress, systemic inflammation, and multi-organ dysfunction represent major clinical outcomes. The parallels between rodent and human responses emphasize the predictive and diagnostic potential of biomarker-based screening for early detection and management of heat-induced organ injury. By identifying reliable biomarkers that reflect the onset and progression of thermal stress, this review contributes to the advancement of non-invasive monitoring tools and therapeutic strategies for mitigating the impact of hyperthermia in both experimental and clinical settings.

At the organ-specific level, renal and hepatic biomarkers provide crucial information. Hyperthermia-induced renal impairment is reflected in elevated creatinine and blood urea nitrogen (BUN) levels, which indicate reduced glomerular filtration and nephron injury (Mei *et al*. 2025). Similarly, liver dysfunction can be observed through altered levels of enzymes such as ALT, AST, and ALP, which are markers of hepatocellular damage and cholestasis (Hasan *et al*. 2018). These biochemical changes correlate well with histopathological findings such as tubular necrosis in kidneys and hepatocyte degeneration in the liver (Vlad *et al*. 2010). Haematological parameters also reveal systemic adaptations and stress responses. Hyperthermia often leads to haemoconcentration due to dehydration, resulting in increased haematocrit and haemoglobin concentration. Changes in leukocyte profiles can indicate an ongoing inflammatory response, while platelet alterations may hint at coagulation abnormalities or endothelial dysfunction (Ajanović *et al*. 2024). Confirmatory evidence of tissue damage is provided by histopathology and apoptosis markers. Histological analysis of hyperthermic organs often shows necrosis, inflammatory infiltrates, vascular congestion, and oedema (Vlad *et al*. 2013). At the molecular level, apoptotic pathways are activated through mitochondrial dysfunction, characterized by upregulation of pro-apoptotic proteins (e.g., Bax), downregulation of anti-apoptotic proteins (e.g., Bcl-2), and activation of caspase cascades. These changes confirm that hyperthermia-induced injury extends beyond reversible stress responses, progressing to irreversible cell death (Sakaguchi *et al*. 1995).

Integration of these diverse biomarkers offers several advantages for experimental and translational research. Firstly, it enables the assessment of severity and duration of hyperthermia, as different markers appear at distinct time points during the progression of stress. Secondly, it helps in determining organ-specific vulnerability, thereby highlighting critical targets such as the brain, liver, and kidneys, which are often most sensitive to heat. Thirdly, biomarker analysis allows researchers to investigate mechanistic pathways, including ROS generation, NF-κB-mediated inflammation, and apoptotic signaling, thereby providing a holistic view of the molecular events underpinning hyperthermia. Finally, these biomarkers can serve as valuable tools to evaluate therapeutic interventions, such as antioxidants, anti-inflammatory agents, or cooling strategies, aimed at mitigating the harmful effects of extreme heat exposure.

Limitations of Current Biomarker Studies

Despite significant progress in understanding the pathophysiological impact of hyperthermia, current biomarker-based studies in male Wistar rats exhibit several important limitations that restrict cross-comparative interpretation and translational application. Most investigations are characterized by small sample sizes and short experimental durations, which limit statistical power and the ability to capture temporal biomarker dynamics during onset, peak, and recovery phases of heat exposure. Moreover, heterogeneity in experimental protocols including variations in ambient temperature (ranging from 38°C to 44°C), exposure duration (30 minutes to 6 hours), recovery periods, and animal housing conditions creates challenges in standardizing results across studies.

Another critical limitation lies in the inconsistent measurement of biomarkers. While many studies report oxidative stress parameters such as MDA, SOD, and

GSH, fewer incorporate molecular or histopathological validations, leading to fragmented datasets that fail to capture the complete pathophysiological sequence. Additionally, differences in analytical techniques (e.g., ELISA, spectrophotometry, or Western blotting) can yield variable outcomes, particularly when laboratory-specific calibration and detection sensitivity differ.

A further constraint is the limited inclusion of multi-organ and multi-omics analyses. Most studies focus on single-organ assessments, such as liver or kidney, neglecting cross-talk mechanisms among organs (brain–liver–kidney axis) that may determine overall systemic vulnerability to hyperthermia. Similarly, the integration of transcriptomic, proteomic, and metabolomic data remains scarce, impeding mechanistic understanding and biomarker validation.

Finally, translational relevance remains underexplored. Although male Wistar rats provide a robust model, interspecies differences in thermoregulatory capacity, metabolic rate, and cardiovascular physiology mean that extrapolation to human heat stress requires caution. Future investigations should thus prioritize standardized protocols, integrative biomarker profiling, and larger cohort studies to enhance reproducibility and bridge the gap between animal models and human heat-related disorders.

CONCLUSION

Male Wistar rats exposed to hyperthermia display a broad spectrum of systemic and cellular responses, reflected in biochemical, oxidative, inflammatory, renal, hepatic, hematological, and tissue-specific biomarkers. Together, these markers illustrate how hyperthermia disrupts metabolic balance, promotes ROS generation, triggers inflammatory cascades, and culminates in organ-specific injury. Integrating such diverse biomarkers provides a comprehensive framework to assess heat-induced stress, evaluate organ vulnerability, and better understand the mechanistic pathways underlying hyperthermia.

Importantly, these insights have translational relevance, as rodent models mirror many clinical features of human heat-related illnesses. Monitoring biomarker profiles may therefore support the development of predictive tools and targeted therapeutic strategies. Future research should emphasize longitudinal analyses, multi-omics integration, and interventional studies to identify novel markers and effective treatments for mitigating heat-induced organ damage.

Significance of this Review

This review consolidates scattered evidence on the systemic response to hyperthermia in male Wistar rats, providing researchers with a comprehensive understanding of the multiple biomarkers involved in heat-induced injury. By integrating biochemical, oxidative, inflammatory, hematological, renal, hepatic, and histopathological markers, it creates a unified framework that can serve as a reference point for designing experiments, interpreting data, and selecting appropriate end points.

For the research community, this review highlights not only the mechanistic pathways of hyperthermia but also the translational potential of these findings in developing diagnostic, prognostic, and therapeutic strategies for heat-related illnesses. It will assist scientists in identifying knowledge gaps, prioritizing research directions, and applying multi-omics and biomarker-based approaches in both preclinical and clinical contexts. Ultimately, it supports the development of standardized protocols and paves the way for innovations in managing hyperthermia-induced morbidity and mortality.

Future Scope

While this review consolidates current knowledge on the systemic and cellular responses to hyperthermia in male Wistar rats, several avenues remain for future exploration. Longitudinal studies are needed to capture the dynamic progression of biomarkers during the onset, peak, and recovery phases of heat exposure. The application of multi-omics approaches, including genomics, proteomics, metabolomics, and transcriptomics, holds promise for identifying novel pathways and biomarker networks that remain unexplored in the context of hyperthermia. Moreover, greater attention should be given to organ cross-talk mechanisms, as the interplay between the brain, liver, kidney, and cardiovascular system likely shapes overall systemic vulnerability. Validation of existing findings in larger cohorts and diverse animal models will strengthen reproducibility and translational relevance. Equally important is the investigation of therapeutic strategies such as antioxidants, anti-inflammatory agents, and cooling interventions using biomarker-based monitoring to assess efficacy. Finally, bridging preclinical data with human studies will be essential to address the growing burden of heat-related illnesses, particularly in the face of global climate change. Collectively, these future directions will enhance our mechanistic understanding of hyperthermia and support the development of predictive, preventive, and therapeutic frameworks.

AUTHORS CONTRIBUTIONS

All authors contributed to the study conception and design. The first draft of the manuscript was written by Anjali Kumari, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The author here declares there is no conflict of interest in the publication of this article.

List of Abbreviations

ALT – Alanine aminotransferase
ALP – Alkaline phosphatase
AST – Aspartate aminotransferase
Bax – Bcl-2–associated X protein

Bcl-2 – B-cell lymphoma 2
BOD – Biological Oxygen Demand
BUN – Blood urea nitrogen
Ca^{2+} – Calcium ion
CRP – C-reactive protein
ECG – Electrocardiogram
ELISA – Enzyme-linked immunosorbent assay
GPx – Glutathione peroxidase
GSH – Glutathione
H&E – Hematoxylin and eosin
HPLC – High-performance liquid chromatography
HSE – Heat shock element
HSF1 – Heat shock factor 1
HSP – Heat shock protein
HSP70 – Heat shock protein 70
HSP90 – Heat shock protein 90
IHC – Immunohistochemistry
IL-1β – Interleukin-1 beta
IL-6 – Interleukin-6
K^+ – Potassium ion
MDA – Malondialdehyde
mTOR – Mammalian target of rapamycin
Na^+ – Sodium ion
NF-κB – Nuclear factor kappa B
PCR – Polymerase chain reaction
PI3K – Phosphoinositide 3-kinase
qPCR – Quantitative polymerase chain reaction
ROS – Reactive oxygen species
SAM – Sympathetic–adrenal–medullary axis
SIRS – Systemic inflammatory response syndrome
SOD – Superoxide dismutase
TBARS – Thiobarbituric acid reactive substances
TNF-α – Tumor necrosis factor-alpha

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