Original Research Article

Effect of subacute heat stress on histophysiology of hepatorenal system in Wistar rats and the ameliorative effect of combined antioxidants: An investigation

ABSTRACT

Aim: To determine the effect of subacute heat stress on function and histological structure of hepatorenal system.

Study design: 60 rats (30 male and 30 female) were divided into 3 groups (N=10 rats/sex/group). Group I, (Control) maintained at a comfortable: 22±3˚C temperature, Group II (Heat stressed group) rats were subjected to heat stress @ 42 ±1°C for 2 hrs daily and group III rats along with exposure to heat stress @ 42 ±1°C for 2 hrs were administered with Vit C, E and Se together daily for 30 days.

Place and Duration of Study: Study was conducted in the Department of Veterinary Pathology and rats were housed in Central Laboratory Animal House, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh (M.P.), India, from April 2024- Aug 2024 (5 months).

Methodology: Serum biochemistry was done at the end of the experiment *i.e.*, on day 30 for parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, blood urea nitrogen (BUN) and creatinine. Rats were humanely sacrificed and the liver and kidney were collected for histopathology.

Results: In Group II both male and female rats showed a significant increase (P≤0.05) in levels of ALT, AST, total protein and BUN compared to control. Group III male and female rats revealed a significant improvement in these parameters. Histopathological examination of liver revealed majorly dilatation and congested blood vessels, apoptotic cells, and vacuolation. In Kidney of group II male and female rats, there was dilatation and congestion in the renal tubules was noticed along with adaptive changes in the tubular cells. In group III male and female rats improvement was noticed on those microscopic changes in organs which were more profound in group II.

Conclusion: Combined administration of antioxidants has a protective role over heat-stressed induced hepatorenal damage.

Keywords: biochemistry, Heat stress, histopathology, kidney, liver and rats

INTRODUCTION

Heat stress is defined as the perceived discomfort and physiological strain associated with exposure to hot environment. It is a type of natural hazard, caused by high ambient temperature especially in summer. In recent years climate change has become a critical matter of concern throughout the world. Reports have mentioned that “likely range” of increase in global average surface temperature between 0.3 °C and 4.8 °C by the year 2100 (Collier *et al*., 2019).

Heat stress is one of the most stressful events in the life of livestock with harmful consequences on animal health, productivity and product quality. Ruminants, pigs and poultry are susceptible to heat stress due to their rapid metabolic and growth rate. High level of production and species-specific characteristics such as rumen fermentation, sweating impairment and skin insulation also contribute in this (Gonzalez *et al*., 2020).

Heat stress causes functional and metabolic alterations in different cells and tissues of various organs. The inability of the body to cope with increased temperatures may result in adverse pathological changes in vital organs at macroscopic and microscopic levels.

High environmental temperatures enhance the efforts to dissipate excessive body heat, which results in increased respiratory rate, body temperature and water consumption, and a decline in food intake (Yadav *et al*., 2015).

Severe heat stress has been reported to affect almost every organ in the body. Earlier researchers have found various negative impacts of heat stress on the hepatic system. As most of the blood flow is diverted towards peripheral circulation for heat dissipation, this causes a fall in the liver oxygen content and a significant increase in the release of liver glucose (Agarwal and Gupta, 2013).

As per the previous reports, acute kidney injury is the common consequence of heat stress as a result of renal parenchyma injury which is caused by dehydration and hypovolemia, direct thermal injury, rhabdomyolysis-associated myoglobinuria, and systemic inflammatory response syndrome (Zhao, 2021).

The mechanism involved in heat-induced tissue damage is oxidative stress, which is attributed to the excessive formation of oxygen-free radicals (Belhadj *et al*., 2016). Micronutrients especially selenium and vitamins (C and E) are well-known antioxidants. Vitamin C and E work in a synergistic role in protecting the tissue from oxidative stress by neutralizing and clearing oxygen-free radicals. Vitamin E neutralize lipid hydroperoxyl radicals and in this process, it gets converted into tocoperoxyl radicals, Vit C causes regeneration of vit E. In this way the combination vit C and E work synergistically to prevent a chain of lipid peroxidation and prevent cell membrane damage. (Traber & Stevens, 2011; Hidayatik *et al*., 2021).

2. MATERIALS AND METHODS

2.1. Animal and experimental design

The study was carried out in male and female albino wistar rats (*Rattus norvegicus*) of 6-8 weeks age. Rats were given *ad-libitum* feed and water. The rats were maintained in environmental conditions like 22±3°C temperature and 12 hours light and dark cycle as per the guidelines of Committee for Control and Supervision of Experiments on Animals (CCSEA). 30 male and 30 female rats were randomly divided into three groups (N=10 rats/sex/group). Rats of group I served as control (CN) were maintained at a temperature 22±3 ˚C. Group II rats were subjected to heat stress (HS) at 42 ± 1˚C temperature for 2 hrs duration, daily for 30 days. Group III were provided with combination of a combination of selenium (Se @ 0.2 mg/kg), vitamin C and vitamin E @ 100 mg/kg body weight each by oral route daily along with exposure to heat stress at 42 ± 1˚C temperature for 2 hrs duration, daily for 30 days. Heat stress was provided by a halogen room heater (1200 watt) set at a temperature of 42±1 ºC, controlled by a safety thermostat connected to the heater to prevent overheating. provided ventilation was provided by a ceiling fan and a humidifier maintained the relative humidity (RH) at 40-45 %.

Comment [M1]: Is room heater used to provide both 42 ºC and 22 ºC..? make it clear

2.2 Serum Biochemistry

Approximately 1 ml of blood was collected on day 30 of the experiment in an aseptic condition through retro-orbital plexuses. The blood samples were collected into two sterilized eppendorf tubes,

Comment [M2]: How many samples were analysed

for serum biochemical analysis. Serum samples were analyzed for biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, blood urea nitrogen (BUN) and creatinine using semi-automatic biochemical analyzer (Make: Erba mannheim) by utilizing commercially available kits (SGOT kit for AST: Erba Mannheim, 120204; SGPT kit for ALT: Erba Mannheim, 120207; Total protein kit: Erba Mannheim,120231; BUN kit: Erba Mannheim,120214; Creatinine: Erba Mannheim,120246).

2.3 Histopathology

At the end of experiment on day 30, both male and female rats from each group were humanely euthanized and subjected to detailed necropsy. kidney and liver were collected in 10% formalin for histopathological examination. Collected tissue samples were fixed in 10 % formalin for 48-72 hrs period then were washed for 10-12 hrs in running tap water then dehydrated and cleared in acetone - benzene series respectively. Tissues were embedded in paraffin wax (58-60 ºC). Section cutting was done at 5 µm thickness and Hematoxylin & Eosin staining was performed as per the method described by Gridley (1960).

Comment [M3]: How many ../ all 10....? Is there any mortality during the experiment..?

2.4 Statistical Analysis

The quantitative data obtained in the experiment are presented in mean ± SE of the groups in tabular format and subjected for one way Anova for between-group analysis.

Comment [M4]: Include reference

Statement of animal rights

The rats were maintained according to the guidelines of CCSEA where environmental conditions like 22±3°C temperature and 12 hours light and dark cycle were provided. The experiment protocol was approved by institutional animal ethics committee (58/IAEC/Vety./2023, Dated: 21.09.2023).

3. RESULTS AND DISCUSSION

3.1 Biochemistry

Male

On day 30th of biochemical examination, male rats exposed to heat stress at 42±1° C for 2 hrs (G II) continuously for 30 days showed, a significant increase (P = 0.000) in level of ALT, AST (p= 0.000), total protein (p= 0.016) and BUN (p= 0.000) compared to their respective control values.

Group III male rats exposed to heat stress and administered with antioxidants revealed a significant decrease in the level of ALT, AST and total protein compared to group II. These values of group III were comparable to group I except AST which was significantly decreasing. Whereas BUN values remained significantly high in group III against the control and comparable to group II male rats. Table 01 represents the results of the biochemical examination in males of different experimental groups

Table 01: Biochemical parameters in male rats of different experimental groups at day 30 (Mean±SE)

Parameter	Group I	Group II	Group III	P value
ALT (u/l)	041.55^{b} ± 2.43	066.32^{a} ± 3.73	050.51^{b} ± 3.21	0.000
AST (u/l)	119.11^{b} ± 3.44	147.64^{a} ± 2.20	101.63^{c} ± 6.88	0.000
Total Protein (g/dl)	009.76^{b} ± 0.38	012.56^{a} ± 0.84	010.61^{b} ± 0.63	0.016
BUN (mg/dl)	010.32^{b} ± 0.78	019.41^{a} ± 0.64	017.80^{a} ± 0.62	0.000
Creatinine (mg/dl)	000.34^{a} ± 0.04	000.37^{a}± 0.03	000.31^{a} ± 0.03	0.430

Female

On day 30th of the experimental period, a significant increase (P=0.000) in serum ALT, AST and BUN (p= 0.019) in group II and ALT (050.98± 1.55 u/l) in group III were observed in female rats as compared to control. However, group III female rats, showed a significant decrease in the level of ALT (050.98 ± 1.55 u/l), AST (111.04 ± 3.62) and BUN (015.87 ± 0.82 mg/dl) as compared to group II. These values were comparable with control rats except for the level of ALT. Table 02 represents the results of the biochemical examination in females of different experimental groups

Table 02: Biochemical parameters in female rats of different experimental groups at day 30 (Mean±SE)

Parameter	Group I	Group II	Group III	P value
ALT (u/l)	043.87^{c}± 0.64	073.31^{a} ± 3.81	050.98^{b} ± 1.55	0.000
AST (u/l)	099.99^{b} ± 5.59	138.45^{a} ± 5.47	111.04^{b} ± 3.62	0.000
Total Protein (g/dl)	010.25^{ab}± 0.58	011.91^{a} ± 0.58	009.52^{b} ± 0.60	0.028
BUN (mg/dl)	014.22^{b} ±0.96	018.51^{a} ± 1.21	015.87^{ab} ± 0.82	0.019
Creatinine (mg/dl)	000.42^{a} ± 0.04	000.48^{a} ±0.05	000.39^{a} ± 0.03	0.380

In present study, both male and female rats subjected to heat stress showed an increase in serum levels of ALT and AST along with BUN and total protein at termination. Our results are in line with the findings of Agarwal and Gupta (2013), Zhang *et al*. (2018), Odo *et al.* (2019 A), Malyar *et al.* (2021) and Zhao *et al*. (2021). ALT and AST are the liver enzymes and biomarkers of the liver damage. Their elevated levels indicate some level of damage in liver. Heat stress causes the increase permeability of cell membrane and ballooning degeneration in hepatocytes. These changes are responsible for increase of these enzymes. Similarly increase BUN and total protein was noted in heat stressed groups, which might be due to the fluid loss, hyperosmolarity and reduced renal clearance (Zhao *et al*., 2021).

Comment [M5]: Include reference

Administration of antioxidants, vitamin C, E and Selenium to the heat stressed rats could have partially preserved the membrane integrity of hepatocytes and blood vessels over the heat stress induced damage. This could be responsible for lowering these biomarkers (Abdelhamid *et al*., 2019).

3.2 Histopathology

3.2.1 Liver

Microscopic examination of liver section from male and female rats of group I (control group) revealed histologically normal architecture consisting of cords of hepatocytes with normal sinusoidal space, central vein and portal triad.

Group II rats subjected to heat stress, microscopic examination of liver section revealed prominent and moderate degree of vascular and degenerative changes in both male and female rats. However the severity of lesions were pronounced in male rats compared to heat stressed group II female rats. Blood vessels especially the central and portal vein showed dilatation in almost all the rats of group II. In a few sections, extravasation of hemolytic fluid into surrounding tissue along with mild inflammatory cell infiltration were noticed (Figure 01). Vacuolar degeneration of hepatocytes was observed (Figure 02). Dilatation and congestion of sinusoids were also clearly evident. Hepatocytes revealed cytoplasmic hypereosinophilia and increased granularity (Figure 04). Increased apoptotic hepatocytes were observed.

Group III rats which were exposed to heat stress and administered with antioxidants like Vit. C, Vit. E and Selenium, also exhibited the vascular and degenerative changes in liver. However, the degree of congestion of blood vessels was more or less the same as that of group II, but degenerative changes

were in milder intensity. Group III rat's liver showed dilatation and congestion of central vein and portal vein with minimal extravasation in male and female rats. Minimal vacuolation (Figure 03) and eosinophilic granulation of hepatocytes observed in group III male and female rats.

Our findings are more or less similar to previous work done by Agarwal and Gupta (2013), Zhang *et al.* (2018) and Malyar *et al.,* (2021). The increase in oxidative stress leads to endothelial cell death making the blood vessel fragile and extravasation of hemolytic fluid to perivascular areas (Yadav *et al.,* 2012). Oxidative stress in response to heat stress plays a crucial role inducing hepatic degeneration and apoptosis through the intrinsic mitochondrial pathway (Lee *et al.,* 2008, Gu *et al*., 2014, Gu *et al*., 2015). The granular and hyper-eosinophilic appearance of hepatocytic cytoplasm perhaps due to protein denaturation as a result of disruption of protein structure due to weakening of hydrogen bonds by increased free energy in the cytoplasm followed by heat stress (Shokry *et al.,* 2024).

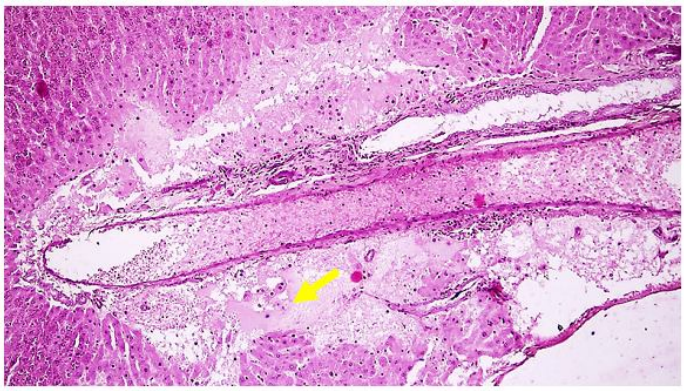

Figure 01: Microscopic section of rat liver (GII, Male) showing dilated blood vessel and extravasation (arrow). H&E X100

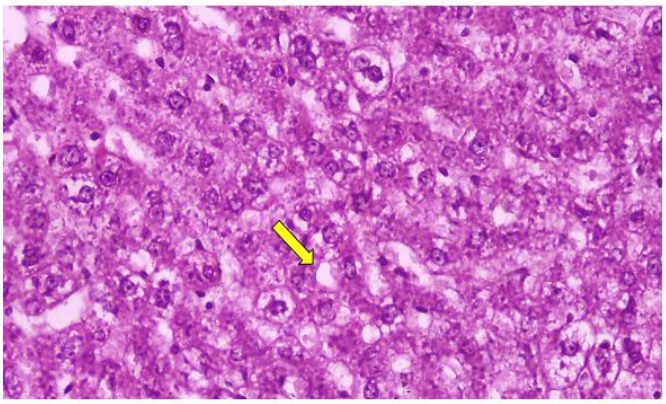

Figure 02: Microscopic section of rat liver (GII, Male) showing hepatocytes with moderate vacuolation (arrow). H&E X400

Figure 03: Microscopic section of rat liver (GIII, Male) showing hepatocytes with minimal vacuolation (arrow). H&E X400

Figure 04: Microscopic section of rat liver (G II, Male) showing hepatocyte with eosinophilic and granulated cytoplasm (arrow). H&E X 400

3.2.2 Kidney

Microscopic examination of group I (control) rats' kidneys both in male and female rats revealed a histologically normal architectural picture comprised of normal renal corpuscles with normal glomerulus, and proximal and distal convoluted tubules in cortex region. The medullary part was comprised of normal loop of Henle and collecting ducts.

Group II rats subjected to heat stress, microscopic examination of kidney section revealed prominent and moderate degree of vascular and degenerative changes in both male and female rats. Kidney of these rats at low magnification showed undulating outer surface, dilatation and congestion of blood vessels in male and female rats. In male rats, dilated vessels and intertubular edematous fluid at corticomedullary junction were quite evident. Glomerulus also showed dilated capillaries. Degenerative changes were evident as dilatation of renal tubules and desquamation of tubular epithelium in the lumen of proximal convoluted tubules (Figure 05). Hypertrophy of occasional tubular epithelium especially in male rats and transitional epithelial cells in pelvis of female rats were evident in some of the sections (Figure 07). Pelvis region also revealed dilated capillaries and renal tubules along with eosinophilic cast (Figure 06)

Group III rats which were exposed to heat stress and administered with antioxidants also exhibited the vascular and degenerative changes in kidney sections of male and female rats. However, the degree of congestion of blood vessels was more or less similar to that of group II, but degenerative changes were in milder intensity. These rats revealed congested blood vessels with no interstitial fluid. Degenerative changes were of milder intensity viz. mild dilatation of tubules and mild desquamation of tubular epithelial cells. The presence of tubular cast was less frequent.

Our findings are in line with previous work done by various researchers (Yadav *et al.* 2015; El-Nager *et al.,* 2019; Zhao *et al*., 2021 and Wang *et al*., 2024). The kidney is an organ sensitive to heat exposure due to its unique function in regulating water-electrolyte metabolism. Heat stress exacerbates load of oxidative stress in tubular epithelial cells which is thought to be the reason for the damage of tubular epithelium. Damaged tubular epithelium results in formation of cast in the tubules. (shokry *et al.*, 2024 and Wang *et al*., 2024).

Figure 05: Microscopic section of rat kidney (GII, Female) showing dilated renal tubules and desquamated epithelium of moderate degree (arrow). H&EX200

Figure 06: Microscopic section of rat kidney (GII, Male) showing dilated capillaries (red arrow) and tubules with cast in pelvis (yellow arrow). H&E X400

Figure 07: Microscopic section of rat kidney (GII, Female) showing transitional cells hyperplasia in pelvis (arrow). H&E X400

4. Conclusion

In conclusion; administration of combined antioxidants Vitamin C and Vitamin E @ 100 mg/kg body weight and Selenium @ 0.2 mg/kg body weight could be used for the alleviation of degenerative changes and restoring function and structure in the liver and kidney caused by heat stress @ 42 ± 1º C for 2hr/day.

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