

Original Research Article

Impact of Subacute Heat Stress on Hepatorenal Histophysiology in Wistar Rats and Protective Role of Combined Antioxidants

ABSTRACT

Aim: This study investigates the impact of subacute heat stress on hepatorenal function and histology in Wistar rats, alongside the protective effects of combined antioxidants (Vitamin C, Vitamin E, and Selenium)

Study design: Sixty rats were divided into control, heat-stressed, and antioxidant-treated heat-stressed groups.

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: Biochemical markers (ALT, AST, BUN, and total protein) and histopathological evaluations revealed significant hepatorenal damage in the heat-stressed group, with marked amelioration in the antioxidant-treated group in both male and female rats.

Conclusion: These findings underscore the therapeutic potential of antioxidant supplementation in mitigating heat stress-induced organ 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 during 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 **hepatorenal** system. As most of the blood flow is diverted towards peripheral circulation for heat dissipation, this causes a fall in the **tissue** oxygen content and a significant increase in the release of liver glucose (Agarwal and Gupta, 2013).

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. The combination of vitamins C and E and selenium has been shown to reduce the detrimental effect of heat stress particularly in poultry (Shakeri *et al.*, 2020). All three work together synergistically protecting the tissue from oxidative stress by neutralizing and clearing oxygen-free radicals. Vitamin C is the regenerator of vitamin E which gets utilized in the process of neutralization of free radicals. Selenium is an essential micronutrient which is required for the effective functioning of the enzyme glutathione peroxidase (Traber & Stevens, 2011; Hidayatik *et al.*, 2021).**

Though climate change is a burning issue in the present scenario, there is a lack of systemic evaluation of its impact on animals and humans. The present experiment on laboratory rats can provide insight into the effect of heat stress on the hepatorenal system, which deals with metabolism, productivity, and health of livestock and humans. Further, the effect of antioxidant agents to mitigate these changes is being evaluated.

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). **A pilot study was conducted for selection of sustainable temperature and duration of heat stress.** 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 under AHU (Air Handling Unit) controlled room Group II rats were subjected to heat stress (HS) at 42 ± 1°C temperature for 2 hrs 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 daily for 30 days. Heat stress was provided by a halogen room heater (1200 watt) set at a temperature of 42±1 °C, controlled by a safety thermostat connected to the heater to prevent overheating. **Ventilation** was provided by a ceiling fan and a humidifier maintained the relative humidity (RH) at 40-45 %.

2.2 Serum Biochemistry

Approximately 1 ml of blood was collected from all **60 rats i.e., 10 rats/group/sex** on day 30 of the experiment in an aseptic condition through retro-orbital plexuses. The blood samples were collected into two sterilized eppendorf tubes, 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 (6/sex/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 °C). Section cutting was done at 5 µm thickness and Hematoxylin & Eosin staining was performed as per the method described by Gridley (1960).

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 (Snedecor and Cochran, 1994).

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 cycles were provided. The experimental 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. **Hepatocytes are abundant sources of ALT and AST. Heat stress results in the generation of enormous amounts of reactive oxygen species (ROS) leading to oxidative stress which causes damaging effects to hepatocytes directly or indirectly. Excessive ROS disrupts the membrane lipid composition through lipid peroxidation which alters the permeability of the cell membrane consequently leading to release of ALT and AST into the bloodstream (Das, 2011; Yang *et al.*, 2024).** 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).

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). 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). **Vitamin C and E work in a synergistic way 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).**

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 specially 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).

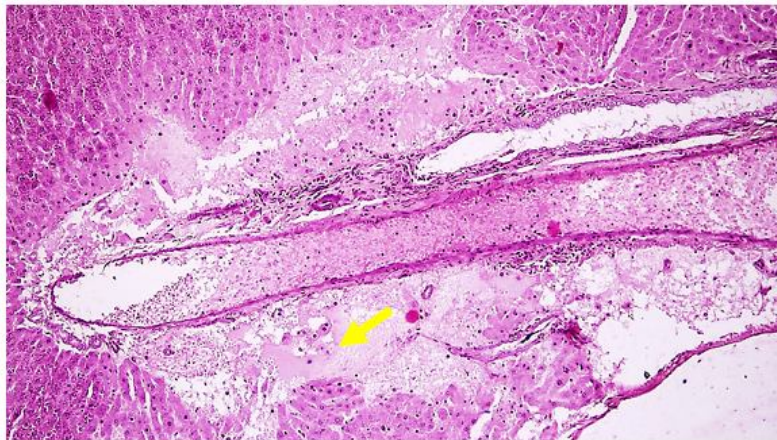


Figure 01: Microscopic section of rat liver (GII, Male) showing dilated blood vessel and extravasation (arrow). H&E X100

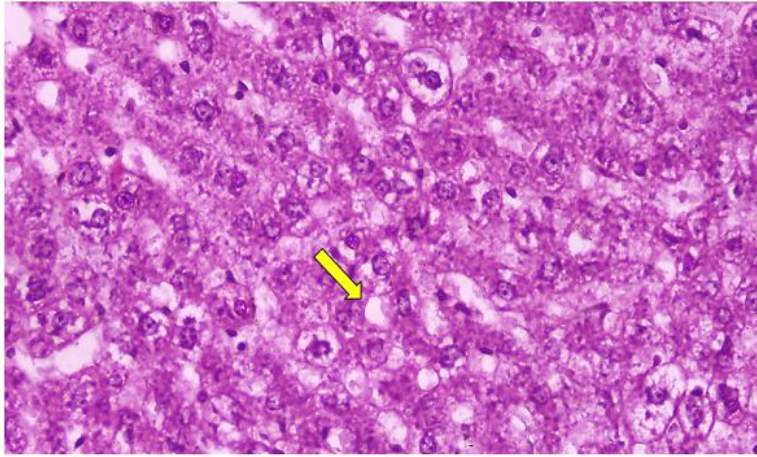


Figure 02: Microscopic section of rat liver (GII, Male) showing hepatocytes with moderate vacuolation (arrow). H&E X400

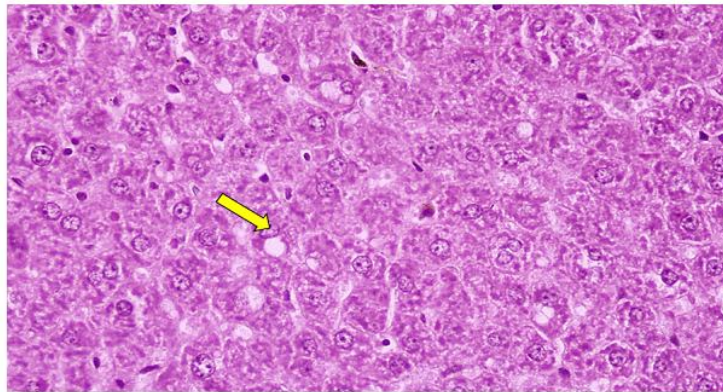


Figure 03: Microscopic section of rat liver (GIII, Male) showing hepatocytes with minimal vacuolation (arrow). H&E X400

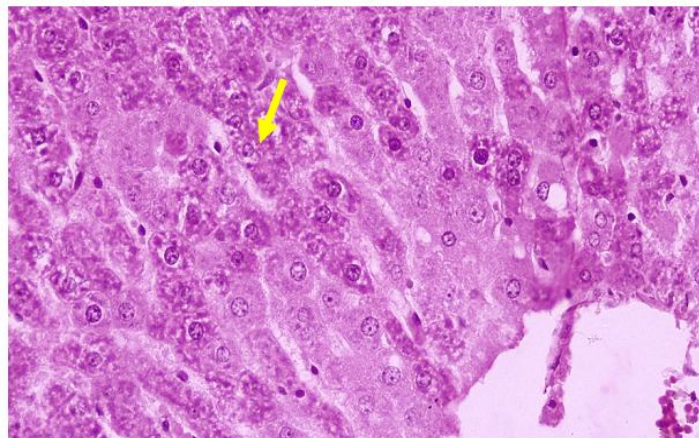


Figure 04: Microscopic section of rat liver (G II, Male) showing hepatocyte with eosinophilic and granulated cytoplasm (arrow). H&E X 400

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).

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).

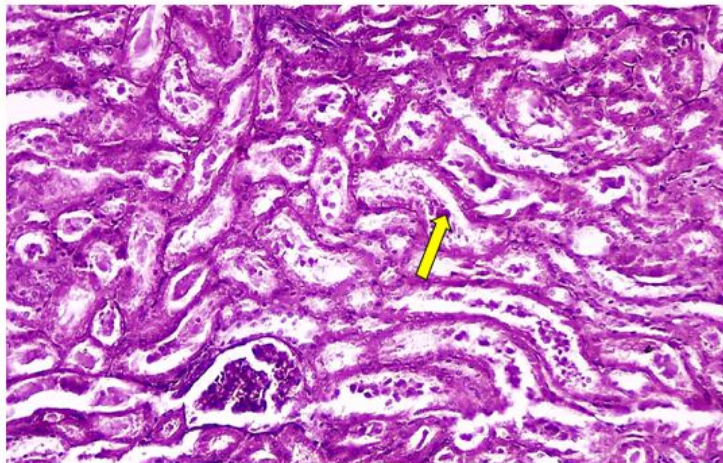


Figure 05: Microscopic section of rat kidney (GII, Female) showing dilated renal tubules and desquamated epithelium of moderate degree (arrow). H&EX200

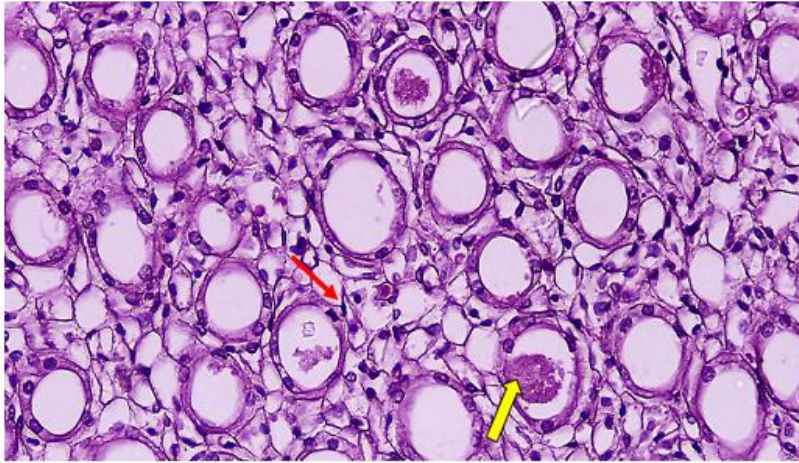


Figure 06: Microscopic section of rat kidney (GII, Male) showing dilated capillaries (red arrow) and tubules with cast in pelvis (yellow arrow). H&E X400

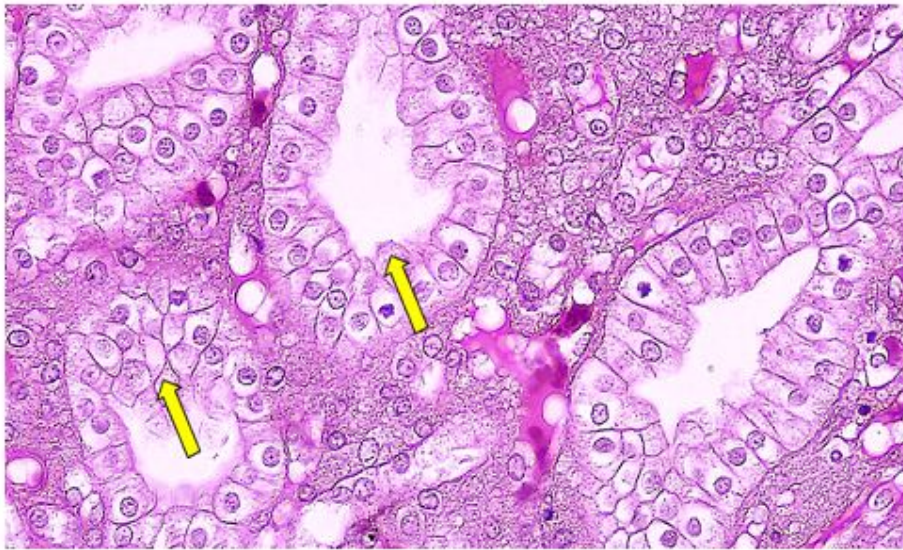


Figure 07: Microscopic section of rat kidney (GII, Female) showing transitional cells hyperplasia in pelvis (arrow). H&E X400

As a consequence of climate change earth's temperature is rising at a concerning rate which is detrimental to overall health and productivity both for animals as well as humans leading to economic loss to the livestock industry and public health care (Shokry *et al.*, 2024). Dairy cattle are more susceptible to heat stress due to high metabolic heat production. In order to dissipate heat from the body, cattle deploy various physiological adaptation mechanisms. One of the major adverse consequences is reduced feed intake and increased susceptibility

to diseases. All these negative impacts result in decreased milk production and decline in fertility (Das *et al.*, 2016). Heat stress has also got deleterious effect on poultry. It disturbs thermoregulation, reduces feed intake and slows growth. It significantly causes damage to gut health which further leads to the malabsorption of nutrients which reduces feed conversion efficiency and growth (Nawaz *et al.*, 2021 and Tang *et al.*, 2022). When it comes to humans then heat stress has got various damaging effects on human health from mild discomfort to severe illness. The supplementation of combination of antioxidants, vitamins C, E and selenium during heat stress has got a protective role against oxidative damage caused by thermal stress both in animals as well as in humans (Abu Hafsa *et al.*, 2024).

4. Conclusion

The present investigation concludes that heat stress caused detrimental effects on the function and histology of the hepatorenal system. However, administration of combined antioxidants Vitamin C and Vitamin E @ 100 mg/kg body weight and Selenium @ 0.2 mg/kg body weight partially alleviated these degenerative changes and restored the function as well as the structure of the liver and kidney caused by heat stress @ $42 \pm 1^\circ \text{C}$ for 2hrs/day. This investigation pointed out that if preventive measures are not taken timely, it can adversely affect the functions of major metabolic organs and the health of the animals as well as human beings. However, detailed molecular investigations are needed to be carried out on a larger population to evaluate the impact in depth.

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Details of the AI usage are given below:

- 1.
- 2.
- 3.

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