

# Protective effects of tocopherol against warm water-induced nephropathy in mice: A biochemical and histological evaluation

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## SUMMARY

Heat stress combined with physical activity can impair kidney function by inducing oxidative damage. Swimming in warm water presents a unique model for studying heat-induced nephropathy. This study evaluated the protective effects of vitamin E (tocopherol) against warm-water-induced renal dysfunction and histological damage in mice. Forty-two male mice were divided into seven groups, including control and experimental groups, with the latter exposed to swimming at either 23°C or 35°C, with or without tocopherol supplementation (100 mg/kg/day). After 35 days, serum, urine and renal samples were analyzed for kidney function markers, oxidative stress indicators and histopathological changes. High-Performance Liquid Chromatography (HPLC) was used for the evaluation of tocopherol accumulation in the renal tissues. Swimming at 23°C caused no significant renal impairment. However, mice swimming at 35°C exhibited increased serum creatinine, reduced estimated glomerular filtration rate (eGFR), elevated malondi-

aldehyde (MDA), decreased superoxide dismutase (SOD) activity, and pronounced tubular necrosis and inflammation indicating acute kidney injury. Tocopherol supplementation in mice swimming at 35°C had beneficial effects on renal function parameters, decreased oxidative stress, and preserved renal histological structure. HPLC confirmed increased tocopherol accumulation in the renal tissues of the supplemented groups. Swimming at 23°C was physiologically safe; however, exposure to 35°C induced measurable renal injury in mice. Tocopherol demonstrated renoprotective effects against heat-induced nephropathy by reducing oxidative stress and maintaining renal structural integrity and function. These findings suggest that tocopherol may serve as a potential therapeutic candidate for individuals exposed to environmental or occupational heat stress.

**Key words:** Hyperthermia – Kidney – Oxidative stress – Swimming – Tocopherol

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## INTRODUCTION

Swimming is widely recognized as an effective form of physical activity for promoting overall health and preventing various illnesses (Tanaka, 2009). It is particularly beneficial due to its engagement of multiple physiological systems, including the cardiovascular and musculoskeletal systems. Moreover, the physical workload during swimming tends to be higher compared to other aerobic exercises (Mingoti et al., 2003). Warm water immersion has been shown to induce muscle relaxation and reduce tension, making it a popular therapeutic modality. However, prolonged exposure to elevated water temperatures may lead to dehydration, which in turn can impair cardiovascular stability and renal function (Pendergast et al., 2015). Moreover, exertional heat injury may result from intense physical activity under hyperthermic conditions, particularly when compounded by muscle damage (Junglee et al., 2013). Both animal studies (Roncal-Jimenez et al., 2015; Roncal-Jimenez et al., 2018), and clinical human research (Junglee et al., 2013; McDermott et al., 2018), have demonstrated that prolonged exposure to heat stress can lead to nephropathy, likely due to repeated episodes of renal injury. The renal system is highly sensitive to thermal and osmotic stress, with kidney function being closely regulated by homeostatic mechanisms and ambient temperature. Moreover, the kidney plays a dual role by not only defending the body against dehydration and thermal load but also being a primary target of heat-related pathologies (Johnson et al., 2019).

Exposure to both clinical and subclinical systemic hyperthermia may exacerbate acute kidney injury (AKI) and trigger inflammatory processes that damage renal tissues (Johnson et al., 2019). Chronic or recurrent heat stress, along with dehydration-induced fluid and electrolyte loss, can result in repeated subclinical ischemic episodes in the kidneys. This condition may ultimately contribute to permanent renal damage and the development of chronic kidney disease (CKD) (Nerbass et al., 2017). Heat stress is also strongly associated with elevated oxidative stress, primarily through the excessive generation of reactive oxygen species (ROS) or the impairment of mitochondrial antioxidant defenses. Prolonged

systemic oxidative stress, induced by sustained exposure to high ambient temperatures, is a recognized contributor to heat-induced renal injury (Belhadj Slimen et al., 2014). Exercising under heat stress may induce mild muscle injury, often reflected by elevated serum creatine kinase levels. These findings are consistent with subclinical rhabdomyolysis, a condition associated with early renal injury markers and impaired kidney function (Roncal-Jimenez et al., 2016).

Antioxidant compounds can be beneficial in mitigating the harmful effects of toxic agents or any activity that generates ROS. The intake of antioxidants enhances the function of the antioxidant defense system. The most effective natural exogenous antioxidants are vitamins and polyphenols (Mohammadghasemi et al., 2021). Tocopherol (vitamin E (VE), a potent lipophilic antioxidant, has demonstrated protective effects against oxidative damage across various organ systems (Błaszczuk et al., 2008). Supplementation with supra-nutritional levels of VE and selenium has been shown to improve immune response and overall physiological resilience in animal models (Chauhan et al., 2014). Warm water swimming has been implicated in nephropathy due to enhanced oxidative stress and reduced renal perfusion (Chapman et al., 2021). The present study investigates whether swimming in warm water induces nephropathy in mice and evaluates the potential protective effects of tocopherol supplementation against heat-induced renal injury.

## MATERIALS AND METHODS

### Ethical statements

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Guilan University of Medical Sciences (Ethics approval number: IR.RUMS.AEC.1400.003).

### Animal model

A total of 42 male BALB/c mice (8-10 weeks old) were obtained from the Animal Facility of the Faculty of Pharmacology, Guilan University of Medical Sciences. Mice were housed in standard ventilated box cages under a 12:12-hour light/dark

cycle with ad libitum access to food and water. All animals were weighed prior to and at the end of the experimental period. The mice were randomly divided into seven groups (n = 6 per group): Group A (Control): No swimming or treatment. Group B (Vehicle): Received sesame oil (solvent) without swimming. Group C (VE only): Received VE without swimming. Group D (23°C): Swimming at 23°C without VE. Group E (23°C + VE): Swimming at 23°C with VE supplementation. Group F (35°C): Swimming at 35°C without VE. Group G (35°C + VE): Swimming at 35°C with VE supplementation (Daeihamed et al., 2024).

Animals in Groups D-G underwent daily swimming sessions in water. Mice were placed in a forced swimming pool (80 cm long, 60 cm wide, and 40 cm deep) filled with tap water for 4 minutes, five days a week, over a period of 5 weeks. The water temperature was kept consistently between 23-24°C and 35-36°C. Animals were deemed exhausted when they could not reach the surface to breathe within 7 seconds and were subsequently removed from the water. Animals in Groups C, E, and G were administered Alpha-tocopherol acetate (VE, Osve-Iran) at a dosage of 100 mg/kg of body weight, which was dissolved in sesame oil and injected intraperitoneally. However, Group C did not participate in swimming, while the animals in Groups E and G did. The treatment duration for the animals receiving VE was identical to that of the other groups, lasting 35 days. In Groups E and G, the VE was injected intraperitoneally one hour prior to swimming.

At the end of the experimental period, all animals were weighed using a digital balance. They were then anesthetized with a combination of ketamine and xylazine (Daeihamed et al., 2024). Blood samples and kidney tissues were collected for subsequent biochemical and histological analyses.

### Urine sample collection

Following the completion of the experimental period, each animal was placed individually in a metabolic glass cage for 24 hours, with free access to food and water. Urine was collected aseptically from the reservoir at the end of the collection period. The total urine volume was recorded, and

aliquots were subsequently stored at -80°C for further biochemical analysis.

### Urine analysis

Renal function was assessed by measuring 24-hour urinary concentrations of creatinine and urea, using colorimetric assay kits (Pars Azmoon, Iran). The estimated glomerular filtration rate (eGFR) was calculated using the following formula:

$$\text{eGFR (mL/min/kg body weight)} = [\text{urinary creatinine (mg/dL)} \times \text{urine volume (mL)} \times 1000] / [\text{serum creatinine (mg/dL)} \times \text{body weight (g)} \times 1440 \text{ (min)}] \text{ (Ren et al., 2022).}$$

### Blood sample collection

Blood samples were collected from the inferior vena cava under terminal anesthesia. The samples were allowed to clot at room temperature, followed by centrifugation at 3,000 × g for 10 minutes to separate the serum. The harvested serum was aliquoted and stored at -80°C until biochemical analysis.

### Biochemical tests of serum samples

Serum levels of albumin, blood urea nitrogen (BUN), and creatinine were measured using commercially available colorimetric assay kits (Pars Azmoon, Iran), following the manufacturer's protocols. All measurements were conducted in duplicate to ensure accuracy and reproducibility.

### Distribution of vitamin E in renal homogenate

VE concentrations in renal tissues were quantified using high-performance liquid chromatography (HPLC), following chromatographic conditions adapted from Rupérez et al. (1998), with slight modifications (Daeihamed et al., 2024). Kidney tissues were carefully excised, weighed, and homogenized in 40 mL of a methanol: water (1:1, v/v) solution. Two sequential extractions were performed using n-hexane as the organic solvent. The resulting extracts were evaporated and reconstituted in 500 µL of methanol, and 100 µL aliquots were injected into the HPLC system. Calibration standards were freshly prepared by spiking pooled blank kidney homogenates with VE

working solutions at concentrations of 10, 20, 50, 75, and 100 µg/mL. Sample preparation procedures for the treatment groups followed the same protocol. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.978 µg/mL and 3.14 µg/mL, respectively (Daeihamed et al., 2024).

### **Kidney histopathology and morphometry**

Kidney tissue samples were fixed in 10% neutral buffered formalin, followed by routine paraffin embedding, sectioning, and hematoxylin-eosin (H&E) staining. From each kidney, three representative sections were prepared, and at least ten random microscopic fields per slide were evaluated under light microscopy at 400× magnification.

Histopathological changes including tubular necrosis, tubular ectasia (dilation), interstitial inflammation or hemorrhage, and vascular congestion were semi-quantitatively scored using the following scale: 0 = none; 1 = <25%; 2 = 25–50%; 3 = 50–75%; and 4 = >75% of the examined field (Gibson-Corley et al., 2013; Song et al., 2019). The percentage of tubules exhibiting brush border loss, necrosis, and ectasia was also recorded in each field.

Representative images were captured using a digital microscope camera connected to a computer. Morphometric measurements of renal corpuscles, glomerular tufts, and Bowman's capsules were performed using Digimizer image analysis software. Both cortical and juxtamedullary nephrons were assessed. A minimum of ten glomeruli per slide (a total of 30 glomeruli per animal) were analyzed to ensure statistical validity (Deji et al., 2009).

### **Renal oxidative markers and antioxidant enzymes**

A portion of each renal tissue sample was rapidly frozen in liquid nitrogen and stored at –80°C for oxidative stress and antioxidant analysis. For biochemical assays, frozen samples were thawed, weighed, and homogenized in ice-cold phosphate-buffered saline (PBS) for 5 minutes. The homogenates were centrifuged at 13,000 × g for 10 minutes at 4°C, and the resulting superna-

tants were collected and stored at –80°C for further analysis. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using a colorimetric assay kit (TPR-MDA; Teb Pazhohan Razi, Iran), with an absorbance read at 545 nm. Antioxidant enzyme activities including catalase (CAT) and superoxide dismutase (SOD) were determined using specific colorimetric kits (TPR-CAT and TPR-SOD; Teb Pazhohan Razi, Iran) at 450 nm. The LODs were 1 U/mL for MDA, 0.5 U/mL for CAT, and 0.1 U/mL for SOD. The total protein concentration in each sample was quantified using the Bradford protein assay method (Faghani et al., 2022).

### **Statistical analysis**

Statistical analyses were performed using GraphPad Prism software (version 10, GraphPad Software Inc., USA). Data are presented as mean ± standard error (SE). The distribution of the residuals was examined using the D'Agostino-Pearson test, while the equality of variances was checked with the Brown-Forsythe test. Based on the outcomes of these initial tests, a suitable statistical method, whether it be one-way ANOVA or the Kruskal-Wallis test was chosen according to whether the assumptions of normality and homogeneity of variances were met. In cases of non-normal data, the Kruskal-Wallis test, was employed. A paired t-test was used to evaluate within-group changes in body weight before and after the experimental intervention. A P-value < 0.05 was considered statistically significant.

## **RESULTS**

### **Animal weights**

As shown in Table 1, all experimental groups exhibited a statistically significant increase in body weight by the end of the study period compared to baseline values (P < 0.05), except Group F (swimming at 35°C), where weight gain was not significant.

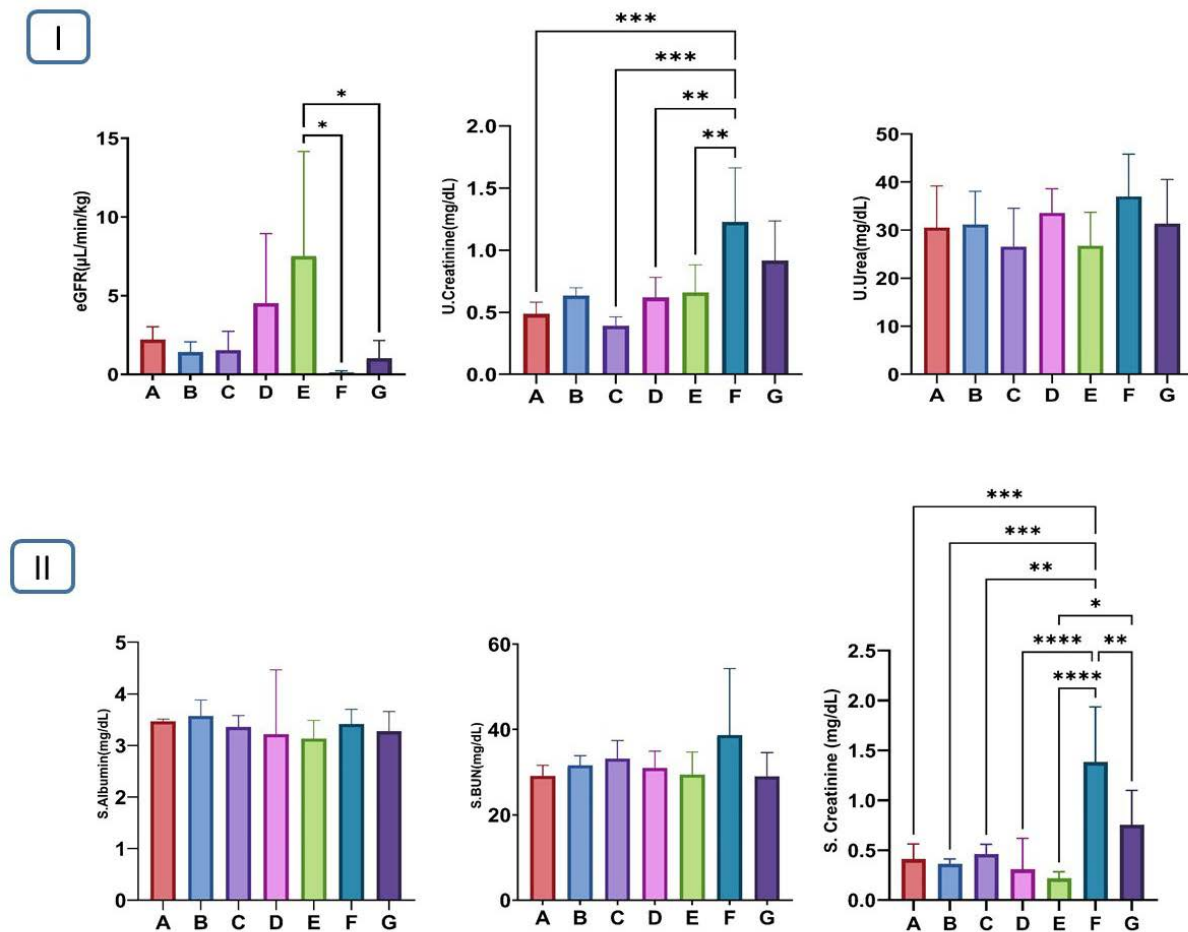
### **Urine analysis**

Urinary creatinine levels in the control group (Group A) were 0.46 ± 0.04 mg/dL (Figure 1, panel I). Group F (swimming at 35°C) exhibited signifi-

**Table 1.** Alterations of animal weights before and after experiments

Groups	A: Control	B: Solvent	C: VE	D: Swimming at 23°C	E: Swimming at 23°C + VE	F: Swimming at 35°C	G: Swimming at 35°C + VE
Weight (g)							
Before experiments	22.75±1.25	26.5±1.89	26.25±2.32	27.25±1.37	24.12±1.85	26.87±1.38	26.62±1.65
After experiments	29.25±2.52	38.75±1.10	37.5±2.10	32.60±0.99	28.87±2.94	26.07±1.97	31.5±1.6
P value	0.05	0.02	0.01	0.001	0.01	0.28	0.01

All data are expressed as mean ± standard error (SE). n=6/group.



**Fig. 1.** - Panel I shows the effects of swimming and receiving VE on urine analysis and eGFR. U: Urinary. Panel II shows the effects of swimming and receiving VE on serum markers of renal function. S: Serum. A: Control, B: Solvent C: VE, D: Swimming at 23°C, E: Swimming at 23°C + VE, F: Swimming at 35°C, G: Swimming at 35°C + VE. All data are expressed as mean ± standard error (SE). \*: P<0.05 \*\*: P=0.001, \*\*\*: P=0.0005 and \*\*\*\*: p=0.0001 n=6/group.

cantly elevated urinary creatinine levels ( $1.22 \pm 0.17$  mg/dL) compared to Groups A–E ( $P < 0.05$ ). Group G (35°C + VE) showed insignificant changes in urinary creatinine ( $0.91 \pm 0.11$  mg/dL) relative to Groups A–F. These findings show a lack of kidney protection capacity of VE in terms of urinary creatinine levels.

Urinary urea levels in the control group were

$30.50 \pm 5.08$  mg/dL. Group F had the highest mean urea concentration among the groups,  $37.00 \pm 3.59$  mg/dL, but the differences were not statistically significant.

The eGFR in the control group was  $2.12 \pm 0.36$  μL/min/kg. A significant decline in eGFR ( $0.12 \pm 0.04$  μL/min/kg) was observed in Group F compared to Group A ( $P < 0.05$ ) and Group E ( $P = 0.0005$ ).

Group G demonstrated a higher eGFR ( $1.02 \pm 0.39$   $\mu\text{L}/\text{min}/\text{kg}$ ) than Group F. The increase was not statistically significant (Fig. 1-panel I).

### Serum's biochemical tests

BUN levels in the control group were  $29.12 \pm 1.23$  mg/dL. No statistically significant differences in BUN levels were observed among the groups ( $P > 0.05$ ).

Serum albumin concentration in the control group was  $3.46 \pm 0.02$  mg/dL, and no significant intergroup differences were detected in this parameter (Fig. 1- panel II).

BUN levels in the control group were  $29.12 \pm 1.23$  mg/dL. No statistically significant differences in BUN were observed among the groups.

Serum creatinine levels in the control group were  $0.41 \pm 0.007$  mg/dL. In Group F ( $1.38 \pm 0.22$  mg/dL), it was significantly higher than in Groups A to E ( $p < 0.05$ ). VE supplementation in Group G significantly reduced serum creatinine ( $0.75 \pm 0.02$  mg/dL) when compared with Groups F and E. There were no significant differences between Groups A to D compared to Group G in Serum creatinine. This shows the protective effect of VE in terms of serum creatinine level in swimming at  $35^\circ\text{C}$  (Fig. 1-panel II).

### Distribution of vitamin E in renal tissue

HPLC analysis revealed that the LOD and LOQ for VE in renal tissue were  $0.978$   $\mu\text{g}/\text{mL}$  and  $3.14$   $\mu\text{g}/\text{mL}$ , respectively. VE levels were below de-

tection limits in Groups A, B, D, and F, which did not receive VE supplementation. In contrast, detectable VE concentrations were observed only in groups that received VE: Group C ( $7.39 \pm 1.69$   $\mu\text{g}/\text{g}$ ), Group E ( $9.34 \pm 1.16$   $\mu\text{g}/\text{g}$ ), and Group G ( $15.85 \pm 2.51$   $\mu\text{g}/\text{g}$ ). Among these, Group G ( $35^\circ\text{C} + \text{VE}$ ) showed the highest renal accumulation of VE, which was significantly greater than both Group C ( $P = 0.0001$ ) and Group E ( $P = 0.0005$ ), as determined by one-way ANOVA followed by Tukey's post hoc test.

### Histopathological findings and renal tissue scoring

In the control group (Group A), histological examination of the renal cortex and medulla revealed normal morphology of renal corpuscles, intact glomerular architecture, and well-organized renal tubules (Figure 2). Similarly, no significant histological abnormalities were observed in the solvent (Group B) and VE-only (Group C) groups. Mild histological alterations were seen in Groups D and E (swimming at  $23^\circ\text{C}$  with/without VE), including limited tubular ectasia or focal epithelial cell desquamation, though these changes were not statistically significant (Fig. 2).

No significant differences were found among Groups D to G in terms of inflammatory cell infiltration ( $P > 0.05$ ). In contrast, severe histopathological damage was observed in Group F (swimming at  $35^\circ\text{C}$ ), including vascular congestion  $\%53.67 \pm 2.66$  vs  $\%19.17 \pm 1.53$ , ( $P = 0.0001$ ), epithelial cell desquamation  $\%0.25 \pm 0.04$  vs  $\%0.01$

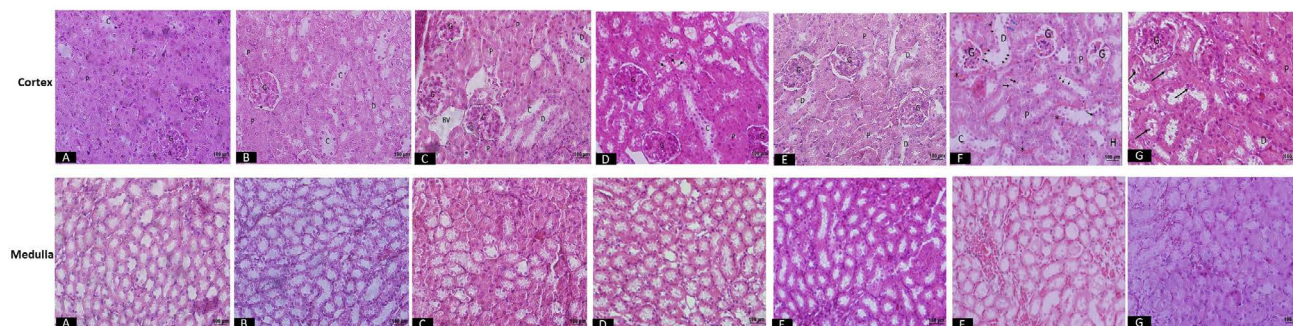


Fig. 2.- Renal histology (cortex and medulla) in a mouse. A: Control, B: Solvent, C: VE, D: Swimming at  $23^\circ\text{C}$ , E: Swimming at  $23^\circ\text{C} + \text{VE}$ , F: Swimming at  $35^\circ\text{C}$ , G: Swimming at  $35^\circ\text{C} + \text{VE}$ . P: Proximal tubule, D: Distal tubule, C: Collecting duct, H: Henle tubule, G: Glomerulus, BV: Blood vessel. Observe the ectasia (wider tubules) in the panel F. Epithelial cell desquamation is shown by long arrows, karyorrhexis is shown by short arrows, and pyknosis is shown by blue arrows. Blood vessel congestion is indicated by signs \*. It is clear that there were no pathological signs in Groups A to C. Take notice of the smaller glomerulus and higher blood vessel congestion in Group F. The preservation of renal histology and congestion could be partially maintained by receiving VE in Group G. Hematoxylin and eosin (400 $\times$ ).

$\pm 0.003$ , ( $P < 0.05$ ), and widespread tubular ectasia  $\%24.17 \pm 1.44$  vs  $\%12.33 \pm 0.84$ , ( $P = 0.0001$ ), when compared with Group G (Fig. 3).

The tissue necrosis percent in Group F ( $0.27 \pm 0.01$ ) was higher compared to Groups D ( $\%0.026 \pm 0.001$ ), ( $P = 0.001$ ) and E ( $\%0.006 \pm 0.00$ ), ( $P = 0.001$ ). Although the level of necrosis in Group G ( $\%0.06 \pm 0.009$ ) decreased compared to Groups D, E, and F, this change was not significant. The ectasia in Group F was also higher than in Groups D and E ( $P = 0.0001$ ). While VE consumption in Group G significantly reduced ectasia compared to Group F, its level remained higher compared to Groups D and E ( $P = 0.0001$ ). Regarding the cell desquamation, no statistical differences were observed between Groups F and D or E. VE consumption in Group G significantly reduced this compared to Group F ( $P = 0.05$ ), while no differences were noted with Groups D and E. Tissue congestion in Group F ( $\%53.67 \pm 2.66$ ) was significantly greater

than in Groups E, D, and G ( $P = 0.0001$ ). Although VE consumption in Group G reduced congestion compared to Group F ( $P = 0.0001$ ), its level remained statistically higher than in Groups E ( $P = 0.0005$ ) and D ( $P = 0.0001$ ) (Fig. 3).

The quantitative morphometric evaluation revealed no significant differences among the groups in cortical renal corpuscle diameter, glomerulus dimension and Bowman's capsule size. However, in the juxtamedullary region, Group F exhibited a marked reduction in renal corpuscle and glomerulus tuft size ( $P < 0.05$ ), along with an increase in Bowman's capsule diameter. VE supplementation in Group G significantly increased the size of juxtamedullary glomerular corpuscles ( $P = 0.001$ ) and glomerulus tufts ( $P < 0.05$ ) compared to Group F, indicating partial recovery of structural integrity (Table 2).

#### Renal homogenate oxidative markers and anti-

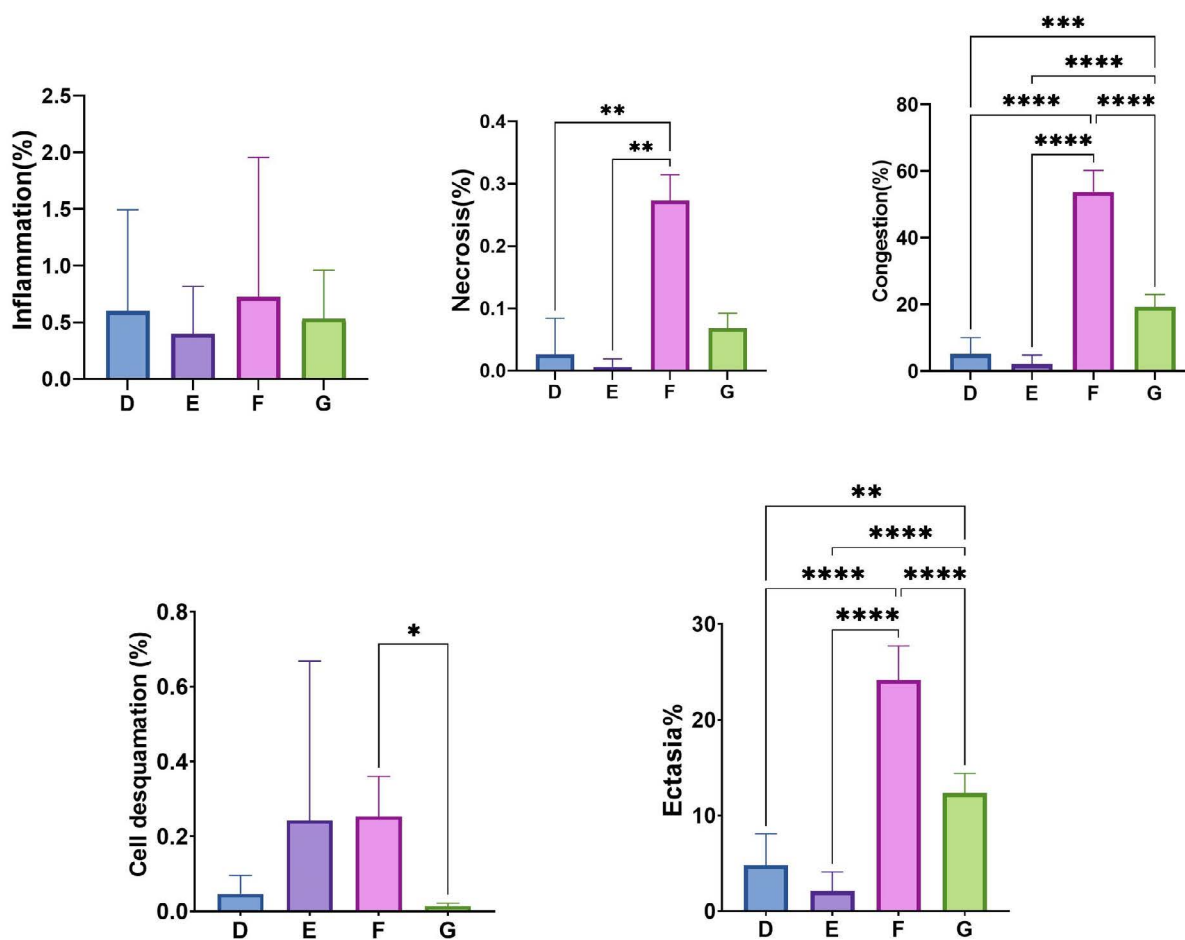


Fig. 3.- The effects of swimming and receiving VE on renal tissue damage. All data are expressed as mean  $\pm$  standard error (SE). Groups A to C were found to have no histological damage. Hence, only groups D to G were analyzed for these signs. D: Swimming at 23°C, E: Swimming at 23°C + VE, F: Swimming at 35°C, G: Swimming at 35°C +VE. \*:  $P < 0.05$ , \*\*:  $P = 0.001$ , \*\*\*:  $P = 0.0005$ , and \*\*\*\*\*:  $P = 0.0001$ .  $n = 6/\text{group}$ .

**Table 2.** Histo-morphometric alterations of renal corpuscles in groups

Groups	A: Control	B: Solvent	C: VE	D: Swimming at 23°C	E: Swimming at 23°C + VE	F: Swimming at 35°C	G: Swimming at 35°C +VE
Co. glomerulus (µm)	200.4 ± 4.36	198.8 ± 4.71	196.75 ± 1.97	192.4 ± 1.72	194.6 ± 1.88	193.1 ± 2.0	194.8 ± 2.85
Jm. glomerulus (µm)	213.6 ± 3.37	208.2 ± 2.76	215.5 ± 3.3	213.2 ± 1.74	208.4 ± 2.92	169.2 ± 4.31a**	191.1 ± 3.4b**
Co. Corpuscle (µm)	210.6 ± 5.98	208.6 ± 5.3	205.25 ± 4.47	201.0 ± 1.94	206.1 ± 7.09	197.4 ± 2.29	200.6 ± 4.23
Jm. Corpuscle (µm)	224.6 ± 5.39	220.8 ± 5.3	216.75 ± 3.75	212.2 ± 1.01	222.8 ± 5.34	178.6 ± 4.22a**	210.52 ± 3.11b*
Co. Bowman space (µm)	13.0 ± 1.09	15.2 ± 0.86	12.7 ± 1.1	12.6 ± 0.92	12.0 ± 1.64	13.6 ± 1.36	14.4 ± 1.02
Jm. Bowman space (µm)	14.6 ± 0.5	16.4 ± 0.74	15.25 ± 1.1	14.8 ± 0.96	15.0 ± 1.26	22.6 ± 0.81a*	18.0 ± 1.14

All data are expressed as mean ± standard error (SE). A: Control, B: Solvent C: VE, D: Swimming at 23°C, E: Swimming at 23°C + VE, F: Swimming at 35°C, G: Swimming at 35°C +VE. Both cortical (Co) and Juxtamedullary (Jm) renal corpuscles are covered by the data. \*:  $p < 0.05$ , and \*\*:  $p = 0.001$ . Significant data are in comparison with the control group. a: in comparison with control and b: in comparison with Group F.  $n = 6$ /group.

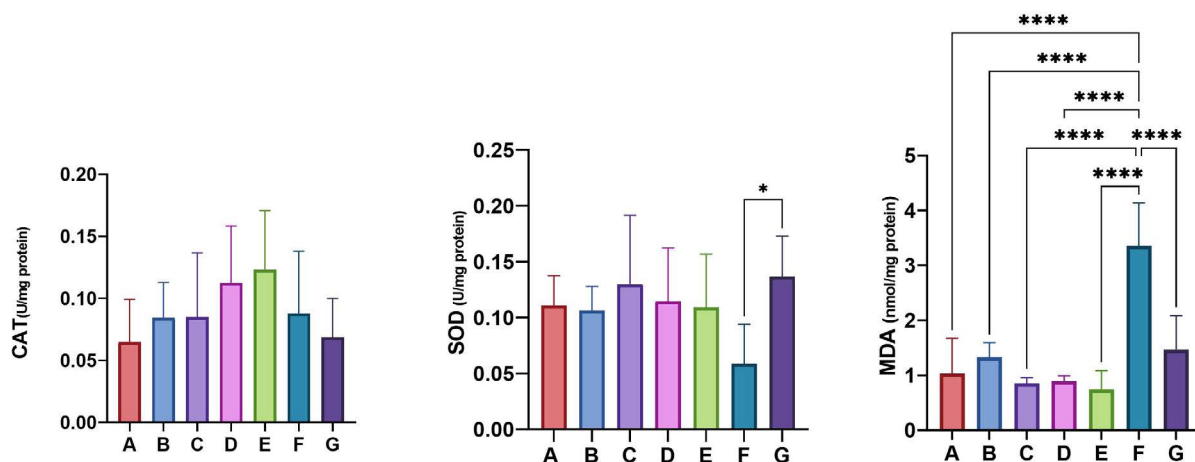
### oxidant enzymes

The CAT activity did not reach a statistical significance between groups. SOD activity was significantly decreased in Group F ( $0.058 \pm 0.014$  U/mg) compared to all other groups. Administration of VE in Group G ( $0.13 \pm 0.01$  U/mg) significantly restored SOD activity compared to Group F ( $P = 0.02$ ). Levels of MDA, a marker of lipid peroxidation, were significantly elevated in the renal tissues of mice in Group F (35°C swimming) compared to all other experimental groups ( $P = 0.0001$ ). VE supplementation in Group G led to

a marked reduction in MDA levels ( $1.47 \pm 0.21$  nmol/mg) relative to Group F ( $3.35 \pm 0.31$  nmol/mg) ( $P = 0.0001$ ), indicating a significant antioxidant effect of VE (Fig. 4).

### DISCUSSION

This study explored how environmental heat stress, physical exercise (via swimming), and VE supplementation interact to affect renal function, histological structure, and oxidative stress in mice. Swimming at 23°C was found to be well-tolerated, with no evidence of renal dysfunction or



**Fig. 4.-** The effects of swimming and receiving VE on stress-induced oxidative markers in renal homogenates. All data are expressed as mean ± standard error (SE). A: Control, B: Solvent C: VE, D: Swimming at 23°C, E: Swimming at 23°C + VE, F: Swimming at 35°C, G: Swimming at 35°C +VE. \*:  $P = 0.02$ , and \*\*\*\*  $p = 0.0001$ ,  $n = 6$ /group.

tissue damage. In contrast, hyperthermic swimming conditions at 35°C produced notable kidney alterations, including elevated serum creatinine, reduced eGFR, increased tubular injury, and higher levels of MDA, an oxidative stress biomarker.

Swimming under hyperthermic conditions (35°C) mimics heat stress scenarios seen in occupational or military environments. Exertional heat stress is known to impair renal function via multiple mechanisms, including hypovolemia, renal hypoperfusion, and mitochondrial oxidative injury (Junglee et al., 2013). This has been reported in sporting activities, military personnel, and other dangerous jobs such as firefighters (Junglee et al., 2013). Serum creatinine, a well-established indicator of glomerular filtration, showed a significant elevation in Group F (mice subjected to 35°C without VE supplementation). This finding is consistent with known pathophysiological patterns of heat-induced AKI. Such injury may result from mechanisms including skeletal muscle breakdown (rhabdomyolysis), systemic dehydration, and ischemic injury to renal tubules, all of which are established contributors to AKI (Kasper, 2004). Strenuous exercise, especially in warm conditions, can temporarily raise serum creatinine levels due to increased creatine metabolism, dehydration, inflammation, and reduced kidney perfusion (Junglee et al., 2013; Wu et al., 2012). Causes of muscle damage after extreme physical activity include sarcomere overstretch, disruption of muscle connective tissue, inflammatory response, metabolic factors (depletion of glycogen stores leads to a lack of ATP and a decreased function of the sarcoplasmic reticulum or sarcolemmal ATPases) and genetic variability. Clinical signs of muscle damage are strength loss, decreased range of motion, swelling and hypertrophy, soreness and an increase of muscle proteins in plasma and other markers such as an increase in plasma myoglobin, creatine kinase, lactate dehydrogenase, troponin I and creatinine (Stožer et al., 2020). Although no exact threshold exists for swimming at 35°C causing permanent kidney damage, repeated exposure to high temperatures may contribute to renal stress and potential harm. Similarly, vitamins E and C and selenium have beneficial effects on creatinine levels

in cadmium-induced renal injury in rats (Karabulut-Bulan et al., 2008).

The lowest eGFR was observed in Group F, which performed swimming under 35°C conditions. Previous studies have shown that exercising under heat stress can exacerbate the decline in glomerular filtration rate (Smith et al., 1952; Yamamoto et al., 2007). Renal blood flow has been reported to decrease during exercise due to redistribution of cardiac output to active muscles (Chapman et al., 2021). While intense exercise can reduce renal perfusion due to hemodynamic shifts, light physical activity may preserve or improve eGFR (Chapman et al., 2021). Exercise-induced dehydration reduces circulating blood volume, thereby impairing renal perfusion and ultimately lowering eGFR. Heat stress and physical exertion stimulate the release of aldosterone and antidiuretic hormone (ADH), which promote sodium retention and water reabsorption. However, these hormonal responses may transiently reduce renal blood flow (Chapman et al., 2021; Pendergast et al., 2015).

Histopathological examination revealed evidence of AKI, including tubular swelling, necrosis, karyorrhexis, inflammatory cell infiltration, and vascular congestion. These alterations are likely mediated by elevated levels of ROS, which contribute to oxidative injury in renal tissues. This oxidative imbalance was confirmed by a significant increase in MDA levels and a reduction in SOD activity in kidney homogenates at 35°C. Furthermore, heat-induced hypoxia and alterations in renal hemodynamics may exacerbate functional impairment, contributing to the progression of kidney injury (Dennis and Witting, 2017). Alterations in renal hemodynamics, such as increased afferent arteriolar resistance or changes in intraglomerular pressure, may contribute to reduced eGFR during heat stress (Hargreaves and Priet, 2018). Oxidative damage to tubular epithelial cells and renal parenchyma is a well-established mechanism underlying AKI (Dennis and Witting, 2017). In animal models, severe acute renal failure has been linked to intensified oxidative stress and diminished endogenous antioxidant capacity (Baliga et al., 1999). Multiple studies in both humans and animals have explored antiox-

idant-based interventions to attenuate the severity of AKI (Chatterjee, 2007; Koyner et al., 2008). Although ROS are essential for cell signaling and physiological regulation, their excessive accumulation is associated with both acute and chronic kidney injuries. Antioxidants play a key role in preserving redox homeostasis in vivo and safeguarding renal tissues from oxidative damage (Dennis and Witting, 2017).

Tocopherol is a lipid-soluble antioxidant that protects cellular membranes from oxidative damage and contributes to cellular integrity (Herrera and Barbas, 2001). Tocopherol has demonstrated protective effects against ROS-induced nephrotoxicity by mitigating oxidative stress in renal tissues (Badgular et al., 2015; Haidara et al., 2009). Other antioxidant agents, including melatonin (Erdemli et al., 2020), zinc (Messaoudi et al., 2009) and selenium (Messaoudi et al., 2009) have also been shown to improve renal function and histological structure in exercise-induced oxidative stress models.

HPLC analysis showed higher renal VE levels in Group G, suggesting effective accumulation during swimming at 35°C. The protective effect may be due to heat- and exercise-induced vasodilation, which likely enhances renal blood flow and promotes efficient VE delivery to kidney tissues (Hargreaves and Priet, 2018). Warm water exercise may enhance lipid metabolism, promoting the release of stored VE from adipose tissue into circulation. Given that VE is stored in lipid-rich compartments like membranes, fat, and the liver, its bioavailability is closely linked to lipid metabolic activity (Herrera and Barbas, 2001). Hyperthermia may enhance VE tissue bioavailability by increasing molecular diffusion across biological membranes (Bunonyo et al., 2023). However, the precise molecular mechanisms underlying this process remain unclear, necessitating further investigation. Histological analysis of kidney tissues from VE-supplemented swimmers revealed reduced signs of inflammation and oxidative injury. These findings suggest that VE contributes to the structural preservation of renal tissues (Bakar et al., 2015; Hussein et al., 2014). Overall, the observed reduction in oxidative and inflammatory markers highlights the antioxidant role of VE in

counteracting heat-induced renal damage (Ozden et al., 2013). VE has shown potential as a renoprotective agent by improving GFR, preserving renal histological structure, and reducing oxidative stress (Ozden et al., 2013).

In this study, we administered 100 mg/kg of VE (Daeihamed et al., 2024), which is equivalent to 1170 mg for humans, based on a conversion rate of 11.7, resulting from 1 mg VE/kg of body weight from mice to males of 70 kg (Springett et al., 2015). Human studies show a daily intake of VE ranging from 24 to 1206 mg without adverse effects (Baltusnikiene et al., 2023). Research on rats and mice indicates that doses from 6.7 to 1000 mg/kg are generally non-toxic (El-Hak et al., 2019; Koya et al., 1997; Trachtman et al., 1996; Zhao et al., 2019), but there is no consensus among rodent studies (Baltusnikiene et al., 2023). Notably, Jansen et al. in 2016 found that 75 mg/kg of VE increased kidney inflammatory biomarkers in mice over six weeks, while a 2018 study showed that 100 to 200 mg/kg for two weeks also raised kidney inflammatory biomarkers in male mice (Jansen et al., 2016, 2018). These variations may be due to factors like the specific form of VE, treatment duration, study design, animal species, and overall health status.

This study has key limitations, including its reliance on a mouse model, lack of long-term follow-up, inconsistent VE dosing and administration, and limited environmental realism. The heat stress simulation at 35°C excluded factors like humidity and sun exposure, reducing ecological validity. Future research should address these gaps to enhance human applicability and improve heat-stress mitigation strategies.

## CONCLUSION

This study indicates that repeated swimming at 23°C, with or without tocopherol supplementation, does not adversely affect renal function in mice. VE has shown renoprotective potential in mice exposed to warm water (35°C), by preserving kidney function, improving histological architecture, and reducing oxidative stress. Further controlled studies are warranted to elucidate the molecular mechanisms by which heat stress

and VE interact to affect renal physiology. Further research is needed to clarify the underlying mechanisms and optimize tocopherol dosing for potential protective strategies in heat-stressed individuals.

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