Kerbala Journal of Veterinary Medical Sciences Issue (3), Volume (2), (2026)

Impact of Experimentally Induced Hyperhomocysteinemia on Oxidative Stress parameters in Adult female Wistar rats

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Received:18/5/2026 **Accepted:** 4/7/2026 **Published:** 6/9/2026

***Abstract*—** Background: Hyperhomocysteinemia (HHCY) is a metabolic disorder characterized by elevated homocysteine (HCY)levels and is considered a risk factor for several pathological conditions. Given the important role of female physiological and hormonal regulation in oxidative homeostasis, this study investigated selected oxidative and antioxidant biomarkers in female rats with experimentally induced HHCY.

Twenty adult female rats were randomly divided into two groups (10/group): a control group and an HHCY group. HHCY was induced by oral administration of L-methionine (500 mg/kg/day) for 21 days. Serum samples were collected at the end of the experiment for biochemical analysis of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and peroxynitrite (ONOO-).

Results: HHCY significantly increased serum MDA and ONOO- levels ($P < 0.0001$) and significantly decreased GSH and SOD levels ($P < 0.0001$) compared with the control group.

In conclusion, Hyperhomocysteinemia alters redox balance by increasing oxidative and nitrosative stress while reducing antioxidant capacity in female rats.

Keywords **—**HHCY L-methionine, Oxidative stress, Antioxidant biomarkers; Female rats.

INTRODUCTION

Homocysteine HCY is an intermediate sulfur-containing amino acid formed during methionine metabolism. Under normal physiological conditions, its concentration is tightly regulated through remethylation and transsulfuration pathways. Disturbance of these mechanisms may result in hyperhomocystenemia, a condition associated with several pathological disorders . Disruption of these metabolic pathways due to genetic, nutritional, or environmental factors may lead to homocysteiene HCY accumulation in the circulation (1,2). Factors contribute development of HHCY, including deficiencies of folate, vit.B6, and vit. B12, alterations in pathway metabolic enzymes such as Methylenetetrahydrofolate reductase (MTHFR), Cystathionine β-synthase (CBS), and methionine synthase. Experimental induction of HHCY by methionine overload is a well-established animal model that produces sustained elevations in HCY levels and allows investigation of its biological consequences (3,5).

Hyperhococysyenemia(HHCY)is associated with many pathological conditions, including neurological, cardiovascular, and metabolic disorders ,also several studies have proposed that oxidative stress represents one of the principal mechanisms underlying HHCY induced tissue injury. Excess homocysteine may creat the generation of reactive oxygen species (ROS), impair antioxidant defense systems, and contribute to cellular dysfunction and tissue damage (6,8).

Although the association between HHCY and oxidative stress has received considerable attention, information regarding its effects in female experimental models remains limited. Studies have focused primarily on male animals, leaving important questions concerning sex-related differences in oxidative responses insufficiently explored. Therefore, the aim of the present to evaluate selected the most important oxidative /antioxidant biomarkers, including malondialdehyde (MDA), nitric oxide (NO), glutathione (GSH), and superoxide dismutase (SOD), in female rats with induced HHCY in femal rats as experimental lab-animal study .

. MATERIALS AND MTHODS

Experimental Animals

Twenty adult female rats weighing 150–190 g were used in present study, were obtained from the Animal House of the College of Pharmacy, University of Kerbala. Rats were housed in standard polypropylene cages under controlled environmental conditions (25 ± 2°C) and 50–60% relative humidity with a 12 h light/dark cycle) and free access to standard laboratory chow and water throughout the experimental period. All animals were adapted for two weeks before the initiation of the experiment(12).

Experimental designé

The animals were randomly allocated into two groups (10/group):

Control Group: animals received a standard diet and distilled water throughout the study period. (HHCY) Group : Rats received L-methionine at a dose of 500 mg/kg body weight/day by oral gavage for 21 daily . The dose was selected

according to previous studies that demonstrated reliable induction of HHCY (5,12).

Collect of the blood samples :

At the end of the experimental period, animals were fasted overnight and anesthetized by chloroform inhalation before sample collection (14). Blood samples were obtained by cardiac puncture using sterile syringes and transferred into plain tubes. Samples were allowed to clot at room temperature and were subsequently centrifuged at 3000 rpm for 15 minutes. The separated serum was collected and stored at −20°C until biochemical analysis (15).

Biochemical Analysis

Serum levels of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and peroxynitrite (ONOO-) were determined using commercially available assay kits according to the manufacturer's instructions. Absorbance was measured using a spectrophotometer, and the obtained values were expressed according to the units specified for each parameter.

Ethical Approval

The study was conducted in the Department of Physiology, College of Veterinary Medicine, University of Kerbala, and was approved under reference number UOK.VET.PH.2025.160.

STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad Prism version 8 and Student's t-test.Data were expressed as mean ± SE. A p-value < 0.05 was considered statistically significant.

RESULT AND DISCUSSION

The present study revealed a significant increase in serum homocysteine levels in the HHCY group 9.00 ± 1.35, compared with the control group 5.00 ± 1.72, (P < 0.0001) (Figure 1).

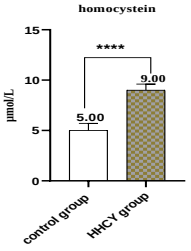

Figure 1. Serum homocysteine levels in control and hyperhomocysteinemic (HHCY) female rats. Values are expressed as Mean ± SE (n = 10). P < 0.0001 compared with the control group**.**

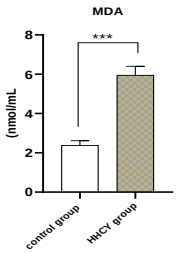

Figure 2. Effect of Hyperhomocysteinemia on Serum Malondialdehyde (MDA) Levels in Female Rats .Values are expressed as Mean ± SE (n = 10). P < 0.0001 compared with the control group.

Figure (2) shows a significant P < 0.0001 increase in the serum MDA levels in the HHCY group6.0 ± 0.4 nmol/mL compared with the control group Control 2.4 ± 0.2 nmol/mL.

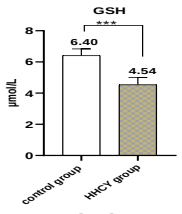

Figure 3. Effect of Hyperhomocysteinemia on Serum Glutathione (GSH) Levels in Female Rats. Values are expressed as Mean ± SE (n = 10). P < 0.001 compared with the control group.

A significant decrease in serum GSH levels was observed in the HHCY group (4.54 ± 0.42) compared with the control group (6.40 ± 0.38) (Figure 3).

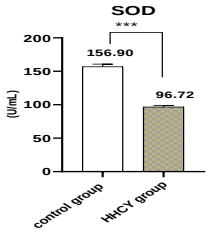

Figure 4. Effect of Hyperhomocysteinemia on Serum Superoxide Dismutase (SOD) Levels in Female Rats. Values are expressed as Mean ± SE (n = 10). P < 0.001 compared with the control group.

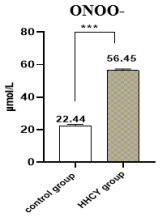

Figure 5. Effect of Hyperhomocysteinemia on Serum Peroxynitrite (ONOO-) Levels in Female Rats.
Values are expressed as Mean ± SE (n = 10). P < 0.001 compared with the control group.

Figure 4 shows a significant decrease in the SOD level in the HHCY group, 96.72 ±4.1 U/mL, compared with the control group 156.90± 3.1. Figure (4), While figure (5) shows a significant increase in the serum (ONOO-I)n the HHCY group56.45 µmol/l ± 1.2, compared with the control group 22.44 ± 2.01.

DISCUSSION

Methionine overload causes a marked increase in the homocysteine levels in the HHCY group compared with the control group. This increase occurs because methionine is the metabolic precursor of homocysteine. If methionine intake leads to an increase in the capacity of remethylation and transsulfuration of methionine metabolism. Homocysteine accumulates in the circulation and increases levels in the serum. This resulted agree with researches have beem noted in experimentally of -methionin overload induced hyperhomocysteinemia, which contributed to elevated circulating homocysteine levels (16,17). This finding is consistent with the metabolic fate of excess methionine responsible for its clearance. (16).

The significant increase in the level of serum (MDA), which was shown in the current study, enhanced lipid per-oxidation and elevated oxidative stress under (HHCY) conditions. (MDA) is a well-recognized end product of polyunsaturated fatty acids per-oxidation and is widely used as indicator unbalance between oxidants and antioxidants in the body. The elevation may be attributed to increased production of reactive oxygen species (ROS) as a result of increased HCY levels, which can induce oxidative stress and membrane damage and compromise cellular integrity. Several mechanisms have been proposed to explain this fact, including (HCY) auto-oxidation, stimulation of NADPH oxidase, mitochondrial dysfunctions, and a decrease in the endogenous antioxidant defenses (18,19).

The results agree with previous experimental studies that noted increased MDA levels in the animals models levels of HCY(20, 21) . Elevated HCY levels were associated with elevation of (ROS)generation and cell membrane per-oxidation and increased MDA serum levels. This concept suggests that oxidative stress represents a major pathogenic consequence of (HHCY).

The current study showed a significant depletion in the serum GSH , which noted to the disturbance in the internal antioxidant levels. HHCY generally causes decreased reduced glutathione (GSH) levels. This occurs because (HCY)metabolism is closely linked to GSH synthesis through the transsulfuration pathway, where homocysteine serves as a precursor to cysteine—the rate-limiting building block for(GSH). 22),

Also, depletion in SOD levels in the present study. As a result of the elevation in HCY levels, the auto-oxidation of (Hcy)generates excess superoxide anions and hydrogen peroxide. To neutralize this overabundance of ROS, SOD and other antioxidants are rapidly consumed, leading to lower measurable enzyme activity in plasma, serum, and tissues (22, 23).

The current study showed an increase in serum Peroxynitrite (ONOO-) levels, suggesting enhanced nitrosative stress in response to HHCY. This contributes to endothelial dysfunction and increases the risk of cardiovascular diseases.(HHcy) stimulates the overproduction of (ROS). Excess superoxide radicals interact directly with (NO), scavenging ii and reducing its biological availability(20,24)

An increase in serum homocysteine has been shown to disrupt cellular redox balance, leading not only to increased (ROS), but also to alterations in nitric oxide metabolism. Elevated (NO) levels may promote the formation of peroxynitrite ($ONOO^-$) by its reaction with superoxide anions, which produce high levels of the ROS capable of stimulating lipid peroxidation, protein modification, and cellular injury (18–21). Experimental studies have shown that increased homocysteine concentrations stimulate inflammatory and oxidative stress that enhance nitric oxide production and contribute to endothelial dysfunction and tissue damage. Therefore, the increased(NO)levels detected in the present study further support the involvement of nitrosative stress as an important component of hyperhomocysteinemia-induced oxidative injury (18–21).

CONCLUSION

Collectively, methionine overload (100 mg/kg/day for 21 days) induced experimental HHCY and disrupted redox homeostasis in female rats. This was evidenced by increased serum homocysteine, (MDA), and(NO)levels, together with decreased(GSH) and (SOD)concentrations. These results indicate enhanced oxidative and nitrosative stress accompanied by depletion of antioxidant defenses, supporting lipid peroxidation as a major mechanism underlying hyperhomocysteinemia-induced tissue injury.

Acknowledgements

The authors would like to acknowledge Department od [physiology, pharmacology and biochemistry of veterinary college and Animal House of the College of Pharmacy, University of Kerbala.

Conflict of interest

All authors declare no conflict of interest .

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