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Histopathological changes in male rats' liver induced by Hyperhomocysteinemia

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***Abstract*—** Homocysteine (Hcy), an amino acid that contains thiols and is naturally formed when methionine and cysteine are exchanged . there is a noticeable rise in Hcy in blood plasma is called hyperhomocysteinemia (HHcy) , which cause cardiovascular disease , cerebrovascular disease and liver tissue destruction . Twenty male rats, participated in the experiment. The rats were split up randomly into HHcy group and control group for 21day of the study , Rats in the HHcy group were given normal diet plus methionine orally (1ml/200g BW) , Rats in control group were given normal diet . At the end of the exposure period, rats was sacrified, and the liver is taken out for histological examination , Haematoxylin and Eosin (Hand E) stain was used to stain the sections . the current study showed a dilated central vein with indistinct hepatocyte swelling and edematous material .

***Keywords* —** hyperhomocysteinemia, liver , rats , edematous material

NTRODUCTION

During methionine metabolism, a substance called homocysteine (Hcy) is created, normal level of Hcy in blood of rats is 5–15 µmol/L (1). when a rise of concentration the Hcy of more than 100 µmol/L HHcy , HHcy is caused by a disruption of the equilibrium between the level of Hcy production and elimination (2). Transsulfation and remethylation activities, in particular, occur in the liver, which plays a crucial role in the production and subsequent metabolism of Hcy (3). It has been established that HHcy can result from harm to organ tissue , In individuals with cirrhosis of the liver (4) . Hcy metabolism abnormalities are also seen , Researchers link this to blocking the production of enzymes, specifically betaine homocysteine methyltransferase, methionine synthase, as well as cystathionine-β-synthase (5) .

Elevated blood homocysteine levels, or hyperhomocysteinemia (HHcy), are strongly linked to an increased risk and severity of non-alcoholic fatty liver disease (NAFLD) , It encourages the buildup of hepatic fat and oxidative stress; research indicates that NAFLD patients, especially those with non-alcoholic steatohepatitis, frequently have much higher homocysteine levels , HHcy causes the liver to experience more oxidative stress, which accelerates the development of NAFLD and contributes to liver damage , Since the liver is the main organ where homocysteine is metabolized, it is especially susceptible to malfunction when levels are high (6) .

Impairments in genetic enzymes, such as MTHFR mutations; vitamin B6, B12, and folic acid shortages; and lifestyle factors, such as heavy alcohol use or smoking, are the main causes of HHcy , It is often associated with aging, certain drugs, and renal failure (7). Studying the histological alterations in the liver tissue of rats with HHcy is the goal of the study .

MATERIALS AND MTHODS

The animal were placed it in good condition in specific plastic cages. Interm of temperature around (30 ±5c), ventilation and the light system was 12 hours per day (8) .Total of 20 male rat, they were placed in the animal House of pharmacy College of Kerbala University , rat were randomly and equally divided into two groups ,each group contain 10 rats received normal diet as control group , and HHcy group animal received normal diet plus methionine (1ml/200g BW) orally daily for 21 day (9) .

At the end of the exposure period, rats was sacrified, and the liver is taken out for histological examination. After the liver was removed right away, they were cleaned with a cooled saline solution (0.9%) , and they were promptly fixed with 10% formalin. They were then treated with xylol and regular alcohol. embedded with paraffin before being divided into pieces. Haematoxylin and Eosin (Hand E) stain was used to stain the sections (10) .

Ethical approval

This investigation was conducted in the physiology department, university of kerbala, college of veterinary medicine under reference number uok.vet.ph.2025.143 .

RESULT

Rat liver histological sections stained with H&E of a control normal group show a highly organised parenchyma, with hepatocytes grouped in discrete plates or cords that are one to two cells thick and extend outward from a central vein toward

the portal tracts, Kupffer cells and endothelial cells line the sinusoids that divide these cords (figure 1) .

According to histological analyses of rat livers of a control normal group showed normal parenchyma with no obvious pathological abnormalities , Hepatocytes radiate from the central vein in neatly ordered plates that are one cell thick , Between the hepatocyte cords are tiny, circulatory pockets called sinusoids , The lobule's central vein (CV) is a thin-walled vein that seems normal and unobstructed , Portal Areas: Typical portal tracts include branches of the hepatic artery, portal vein, and bile duct; occasionally, there are small, dispersed inflammatory cells (figure 2) .

According to histological analyses of rat livers with induced (HHcy) , The central veins (CV) may include proteinaceous fluid or edematous material, and they appear significantly dilated , Hepatocytes exhibit "cloudy swelling," which is defined by larger cells with granular, vacuolated, or pale cytoplasm due to water buildup (cellular edema) , Erythrocytes, leukocytes, and enlarged Kupffer cells frequently fill dilated blood sinusoids , The extreme swelling and parenchymal injury frequently cause the typical radial arrangement of hepatocyte plates (cords) to be altered or fragmented (figure3) .

According to histological analyses of rat livers with induced (HHcy), Oedematous Material/Hydropic Degeneration: The term "oedematous material" describes swelling of the intercellular spaces brought on by non-lethal cell damage or intracellular water buildup (hydropic degeneration) , Ion and fluid balance disruptions cause cloudy swelling (hepatocellular), which is characterized by pale, granular, and larger hepatocytes with cloudy borders (cytoplasmic degeneration) , Hepatic Architecture Distortion , Because of cellular swelling, the typical hepatic cords are frequently disrupted, with sinusoidal gaps exhibiting constriction or blockage (figure 4) .

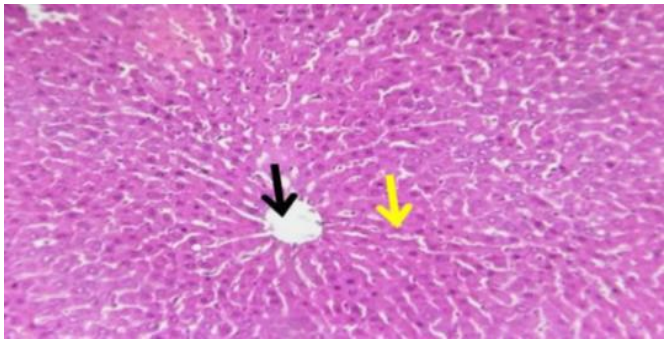

Figure 1. Histological section of liver in a rat (control group) showing the normal lobular architecture and the hepatocytes (yellow arrows) spread as a cord around the central hepatic vein(black arrow). H&E stain.100X

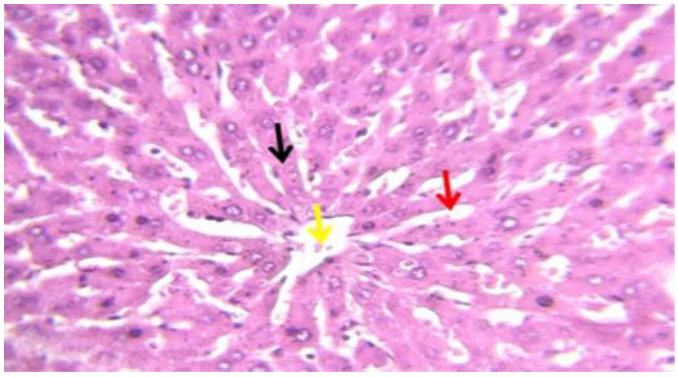

Figure 2. Histological section of liver in rat (control group) showing no clear pathological lesion H&E stain.400X.

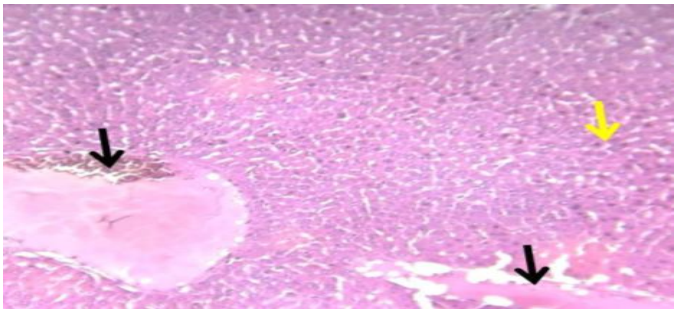

Figure 3 .Histological section of liver in a rat (HHCY group) showing the expanded central vein with odematous material (black arrow) with cloudy swelling of hepatocellular (yellow arrows). H&E stain.100X

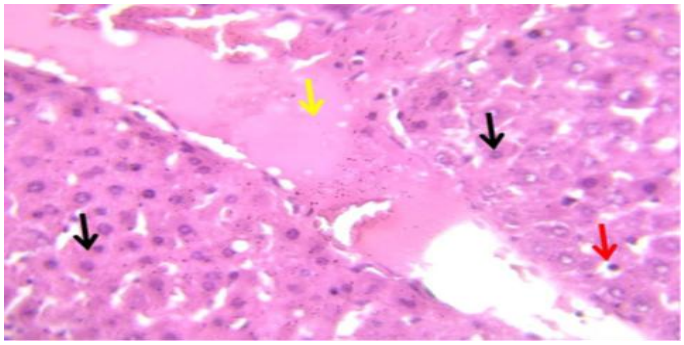

Figure 4. Histological section of liver in rat (HHCY group) showing odematous material with cloudy swelling of hepatocellular (black arrow). H&E stain.400X .

DISCUSSION

Depending on the current study to histological analyses of rat livers with induced (HHcy) showed Hepatocyte swelling (hydropic degeneration), fatty degeneration, localized necrosis, congestion in the portal region, the fibrous connective tissue surrounding the portal veins, and thickening of the portal tracts . HHCY causes the organ's tissue to develop fibrosis (11).

Although the exact mechanisms of this process are still being investigated, the researchers have found that HHcy stimulates the expression of tissue inhibitor of

metalloproteinase-1 (TIMP-1), which leads to the accumulation of procollagen I in hepatocytes and the accumulation of collagen in the liver tissue (12) . Studies conducted by Ukrainian and international researchers demonstrate the strong correlation between liver disease and the blood plasma Hcy level (13) .

The part that HHcy plays in the onset of hepatic steatosis is currently the subject of intense research. Through rat research, it was demonstrated that a complex network of nuclear transcription factors and enzymes is involved in the control of lipogenesis in addition to the recognized mechanisms of lipid accumulation in the organ (14). shown that the presence of HHcy resulted in a considerable increase in the activity of the transcription factor SREBP (sterol regulatory element-binding protein) (15) .

Furthermore, experts note that ER stress is one of the possible causes of fatty infiltration of the liver under these conditions because it is where sterols and lipids are generated in the hepatocyte , There are theories that when there is HHcy in the ER, the expression of genes controlling lipogenesis is increased and the processes of protein synthesis and post-translational modification are greatly suppressed because chaperone activity is inhibited (16). When L-Hcy thiolactone levels in rats rise, hepatocytes experience oxidative stress and their antioxidant defense reserves are depleted, according to research by M. Stojanovic et al.

In the liver tissue of lab animals, the scientists observed a decrease in catalase activity , Consequently, a notable rise in the H2O2 concentration was noted, HHcy was also linked to an increase in the organ's lipid peroxidation processes and the buildup of peroxidation products including thiobarbituric acid (17). Direct harm to the liver cells, their apoptosis, and the start of inflammatory processes were all associated with Lipid peroxidation .

CONCLUSION

1. The amount of connective tissue components in the liver's portal tracts and surrounding the lobules increased in rats with HHcy.

2. Hyperhomocysteinemia caused liver plate rupture, alterations in the nuclear apparatus of hepatocytes, the emergence of hydropic and fatty dystrophy, patches of necrosis in the organ's tissue, and modifications to the vascular structure.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1) Kumar, A., Palfrey, H. A., Pathak, R., Kadowitz, P. J., Gettys, T. W., & Murthy, S. N. (2017). The metabolism and significance of homocysteine in nutrition and health. *Nutrition & metabolism*, *14*(1), 78.

2) Khudiar, K. K., & Al-Bazii, W. G. (2014). Protective role of olive oil and/or folic acid on some biochemical biomarkers related to cardiac disease in Rabbits exposed to Methionine overload. *International Journal of Development*, *3*(2), 67–81.

3) Chen, N. C., Yang, F., Capecci, L. M., Gu, Z., Schafer, A. I., Durante, W., ... & Wang, H. (2010). Regulation of homocysteine metabolism and methylation in human and mouse tissues. *The FASEB Journal*, *24*(8), 2804.

4) Bhanji, R. A., Ma, M., Bain, V. G., & Montano-Loza, A. J. (2016). Hyperhomocysteinemia is associated with severity of cirrhosis and negative impact after liver transplantation. *Liver International*, *36*(5), 696-704.

5) Zaric, B. L., Obradovic, M., Bajic, V., Haidara, M. A., Jovanovic, M., & Isenovic, E. R. (2019). Homocysteine and hyperhomocysteinaemia. *Current medicinal chemistry*, *26*(16), 2948-2961.

6) González-Lamuño, D., Arrieta-Blanco, F. J., Fuentes, E. D., Forga-Visa, M. T., Morales-Conejo, M., Peña-Quintana, L., & Vitoria-Miñana, I. (2023). Hyperhomocysteinemia in adult patients: a treatable metabolic condition. *Nutrients*, *16*(1), 135.

7) Herrmann, W., & Herrmann, M. (2022). The controversial role of HCY and vitamin B deficiency in cardiovascular diseases. *Nutrients*, *14*(7), 1412.

8) Andersson, M., Pernold, K., Lilja, N., Frias-Beneyto, R., & Ulfhake, B. (2024). Longitudinal Study of Changes in Ammonia, Carbon Dioxide, Humidity and Temperature in Individually Ventilated Cages Housing Female and Male C57BL/6N Mice during Consecutive Cycles of Weekly and Bi-Weekly Cage Changes. *Animals*, *14*(18), 2735.

9) Derouiche, F., Djemil, R., Sebihi, F. Z., Douaouya, L., Maamar, H., & Benjemana, K. (2024). High methionine diet mediated oxidative stress and proteasome impairment causes toxicity in liver. *Scientific Reports*, *14*(1), 5555.

10) Maievskyi, O. Y., & Halahan, Y. V. (2023). Microscopic and histochemical changes in the liver of adult rats with hyperhomocysteinemia. *Reports of Morphology*, *29*(1), 4-8.

11) García-Tevijano, E. R., Berasain, C., Rodríguez, J. A., Corrales, F. J., Arias, R., Martín-Duce, A., ... & Avila, M. A. (2001). Hyperhomocysteinemia in liver cirrhosis: mechanisms and role in vascular and hepatic fibrosis. *Hypertension*, *38*(5), 1217-1221.

12) Torres, L., García-Trevijano, E. R., Rodríguez, J. A., Carretero, M. V., Bustos, M., Fernández, E., ... & Avila, M. A. (1999). Induction of TIMP-1 expression in rat hepatic stellate cells and hepatocytes: a new role for homocysteine in liver fibrosis. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1455*(1), 12-22.

13) Halahan, Y. V. (2023). Histological and histochemical changes in liver tissue of young rats with hyperhomocysteinemia. Reports of Vinnytsia National Medical University, 27(1), 6-9.

14) Yao, L., Cao, B., Cheng, Q., Cai, W., Ye, C., Liang, J., ... & Zhu, Y. (2019). Inhibition of soluble epoxide hydrolase ameliorates hyperhomocysteinemia-induced hepatic steatosis by enhancing β-oxidation of fatty acid in mice. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *316*(4), G527-G538.

15) Woo, C. W., Siow, Y. L., Pierce, G. N., Choy, P. C., Minuk, G. Y., Mymin, D., & O, K. (2005). Hyperhomocysteinemia induces hepatic cholesterol biosynthesis and lipid accumulation via activation of transcription factors. *American Journal of Physiology-Endocrinology and Metabolism*, *288*(5), E1002-E1010.

16) Min, Y., Zhang, Y., Ji, Y., Liu, S., Guan, C., Wei, L., ... & Zhang, Z. (2025). Post-translational modifications in the pathophysiological process of metabolic dysfunction-associated steatotic liver disease. *Cell & Bioscience*, *15*(1), 79.

17) Xie, D., & Shi, X. (2025). Lipid peroxidation-induced neurocardiac dysfunction in critically ill patients with septic shock. *Archives of Physiology and Biochemistry*, 1-21.