ISSN: 2980-3063 (online) Original Article

Rats
2023; 1(2): 47-50
Doi: 10.5281/zenodo.10444706
https://ratsjournal.com

Effects of hydrogen-enriched water on lipid profile and some biochemical markers

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Recevied: 25.12.2023 Accepted: 28.12.2023 Published: 30.12.2023

Abstract
Molecular hydrogen (H2) is a non-toxic, tasteless, colorless, and odorless gas with high diffusion properties in living tissues and cells . By scavenging dangerous free radicals like the hydroxyl radical (•OH) and peroxynitrite (ONOO-) within cells, H2 functions as a selective antioxidant. It was aimed to determine the usability of molecular hydrogen (H2) as a new 'pro-metabolic' agent that may affect liver health. The study was initiated with the permission of 2023-154 obtained from Kafkas University Animal Experiments Local Ethics Committee. A total of 14 4-month-old male Wistar Albino rats weighing 200-250 grams, with seven rats in each group, were used in the study. Rats were fed ad-libitium throughout the study. The rats were housed in a 12-hour light and 12-hour dark environment at appropriate temperature. The Unpaired t-test was analyzed for each biochemical parameter to determine the difference between the two groups. A significant P-value was defined as less than 0.05. GraphPad 9.5.1 (San Diego, CA, USA) was used for statistical analysis. Results showed that AST, ALT, total protein, total cholesterol, total cholesterol, and LDL were significantly lower in the H2 group compared to the control group ($P<0.05$). There was no statistically significant difference between the group given H2 and the control group between HDL and TG ($P>0.05$). In conclusion, We think that H2 suppresses oxidative stress, reduces TG, TC, and LDL, which are responsible for vascular occlusion, and can be used for treatment purposes for the liver and many systems and tissues in the future.
Keywords: *Molecular hydrogen, antioxidant, biochemical parameter*

1. Introduction

Molecular hydrogen (H2) is a non-toxic, tasteless, colorless, and odorless gas with high diffusion properties in living tissues and cells.[1] By scavenging dangerous free radicals like the hydroxyl radical (•OH) and peroxynitrite (ONOO-) within cells, H2 functions as a selective antioxidant.[2,3] Its cytoprotective actions have demonstrated positive therapeutic advantages in treating many diseases against oxidative stress.[1,2,4] According to studies on animals, hydrogen can boost the function of the antioxidant system and lessen the harm that oxidative stress does to cells and tissues.[5] Studies showed that in the presence of H2, the cell could prompt the gene expression of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase.[6,7]

Reactive oxygen species (ROS) possess many crucial physiological roles in numerous systems. However, excessive ROS generation can cause cellular macromolecule damage, which ultimately results in cell death. Free radicals can damage phospholipids in cell membranes due to lipid peroxidation. Malondialdehyde (MDA), a by-product of lipid peroxidation, is the frequently used marker to measure lipid peroxidation.[8] A study reported that the leakage of LDL-C into the sub-endothelial space and its oxidation, Ox-LDL, causes pathological conditions such as atherosclerosis.[9] Based on this information, Ox-LDL is a particle derived from circulating LDL with peroxides or their degradation products produced within the LDL molecule or elsewhere in the body associated with LDL fractions. It includes minimally oxidized LDL, which has Ox-LDL and their degraded fraction products but is unaltered concerning apoprotein, MDA-altered MDA particles originating from platelets or elsewhere. However, LDL particles with unaltered lipid content and oxidized apo B amino acids have not been identified.[10]

Molecular hydrogen (H2) has recently emerged as a novel 'pro-metabolic' agent that may positively impact liver health. In addition, drinking hydrogen-rich water (HRW) reduces hepatic oxidative stress and attenuates fatty liver injury in rodents.[11]

2. Material and methods

The study was initiated with the permission of 2023-154 obtained from Kafkas University Animal Experiments Local Ethics Committee. A total of 14 4-month-old male Wistar Albino rats weighing 200-250 grams, with seven rats in each group, were used in the study. Rats were fed ad-libitium throughout the study. The rats were housed in a 12-hour light and 12-hour dark environment at appropriate temperature.

2.1. Study groups

1st group (Control): Group given 5ml/kg distilled water ip for 10 days

How to cite this article: Karapehlivan M, Başer Ömer F, Dolanbay T, Alwazeer D. (2023). Effects of hydrogen-enriched water on lipid profile and some biochemical markers. Rats, 2023; 1(2): 47–50. Doi: 10.5281/zenodo.10444706

2nd group (Hydrogen group): Group given 5ml/kg hydrogen-rich water ip for 10 days

At the end of the study, rats were euthanized under anesthesia (ketamine hydrochloride (75 mg/kg) and xylazine (15 mg/kg) intramuscularly) by cervical dislocation method, and blood samples were collected. The blood samples were centrifuged at 3000 RPM, and the separated sera were stored at -20 C° until analysis.

2.2. Biochemical analysis

ALT, AST, Total Protein, TG, Total Cholesterol, HDL-C, and LDL-C parameters were measured in serum samples. The measurements were spectrophotometrically performed in the Kafkas University, Faculty of Medicine, Beckman-Coulter AU5800 autoanalyzer biochemistry laboratory.

2.3. Statistical analysis

The Unpaired t-test was analyzed for each biochemical parameter to determine the difference between the two groups. A significant P-value was defined as less than 0.05. GraphPad 9.5.1 (San Diego, CA, USA) was used for statistical analysis.

3. Results

Results showed that AST, ALT, total protein, total cholesterol, total cholesterol, and LDL were significantly lower in the H2 group compared to the control group (P<0.05).

There was no statistically significant difference between the group given H2 and the control group between HDL and TG (P>0.05).

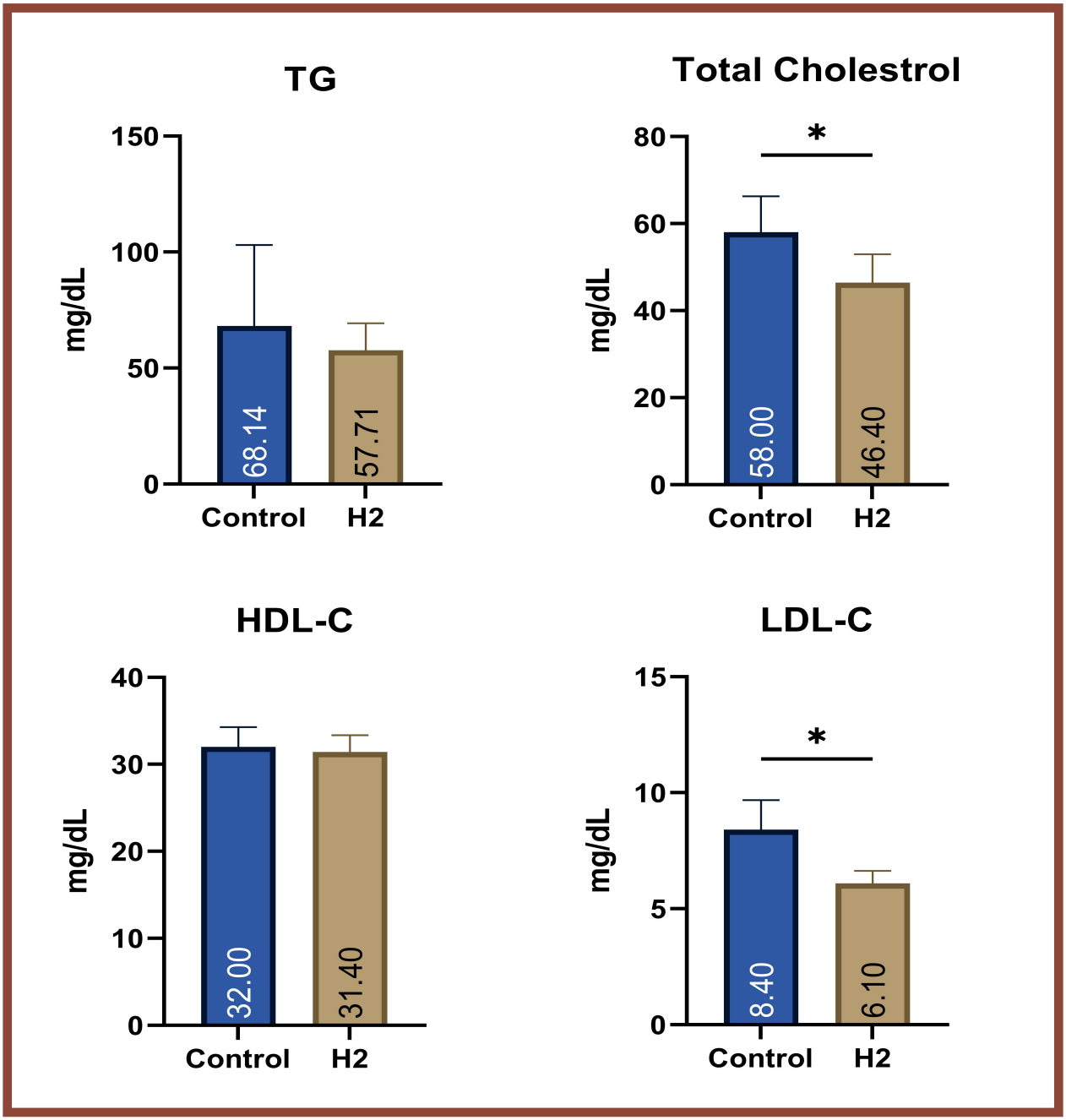

Figure 1. Means and std. errors of the two groups for TG, Total Cholesterol, HDL-C, and LDL-C parameters. *p<0.05

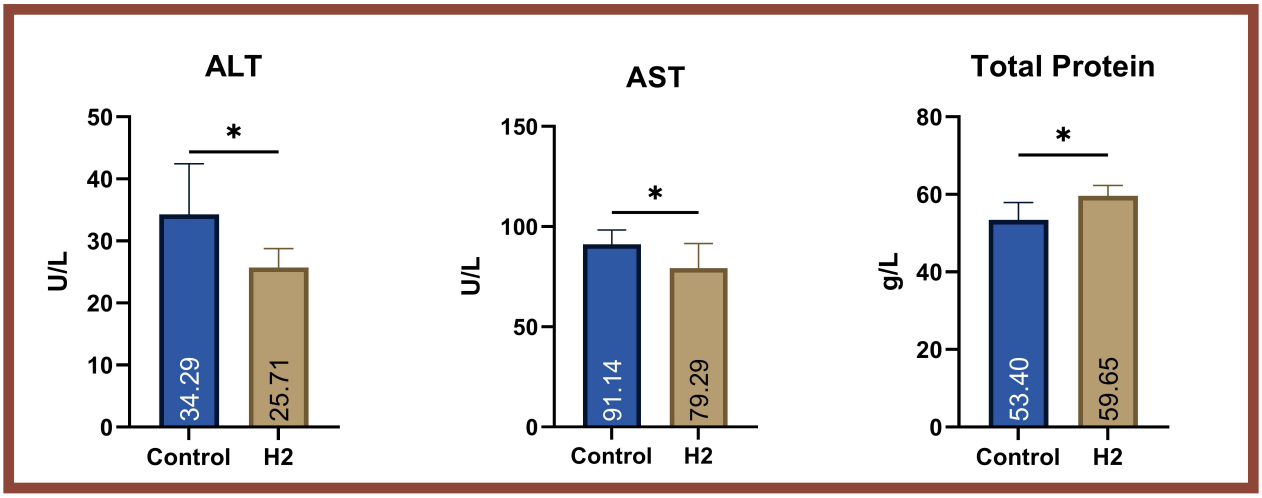

Figure 1. Means and std. errors of the two groups for ALT, AST and Total Protein parameters. *p<0.05

4. Discussion

Under normal conditions, there is a balance between the levels of oxidants, e.g., reactive oxygen radicals, and protective antioxidants. Disruption of this balance between antioxidants and oxidants in favor of oxidants is known as oxidative stress.[12,13]

If the organism's defense mechanisms (antioxidant) against oxidative stress are insufficient, oxidative damage develops in the cells, significantly decreasing cellular functions. Since it has a critical importance in the etiology and pathogenesis of many diseases, the severity of the disease increases. This mechanism is responsible for the aging process and the etiology of many diseases, such as cardiovascular diseases, cancer, sepsis, degenerative neurological diseases, renal failure, infertility, and muscle and liver diseases.[14,15]

ROS causes lipid peroxidation by specifically affecting the unsaturated fatty acids in the cell membrane. Lipid peroxidation is a harmful chemical chain reaction that changes the membrane lipid structure by removing a hydrogen atom from the fatty acid chain in the membrane structure, initiated by free radicals, and indirectly damages the structure and functions of other cell components by producing reactive aldehydes. Once this reaction starts autocatalytically, it continues as a chain. If it is not prevented, it destroys the cell membrane, breaks down organelles, and may cause the release of lysosomal enzymes and autolysis. With this disrupted mechanism, TG cholesterol LDL ratios increase in living things.[11,15–17]

The most crucial liver enzymes are considered to be aminotransferases (AST and ALT) and alkaline phosphatase. These enzymes play a vital role in the early identification of lower liver disorders, in their differential diagnosis, in determining the severity and prognosis of the disease, and in evaluating the treatment approach. The excessive elevation is one of the most critical indicators of acute hepatocellular cell damage in the clinic.[18] Ast in fatty liver lower values increase and the structure of the cells changes, while the fat ratio increases, the protein ratio decreases.[19,20]

Molecular hydrogen (H2) is also a new 'pro-metabolic' agent that can positively affect liver health. Our study showed a decrease in the H2-administered group, especially in the ast sub-values, compared to the control group, and a significant increase in total protein ratios compared to the control group. We think H2 can effectively prevent liver pathologies by reducing oxidative stress and increasing the protein ratio by further reducing the lower liver values evaluated within the normal reference range in blood parameters in healthy individuals. It is known that for oxidative stress, lipid oxidation increases, whereas the antioxidant system weakens. Disorders in carbohydrate and lipid metabolism induce oxidative stress through many unknown mechanisms. LDL is responsible for pathological conditions such as atherosclerosis due to its invasion under the vascular endothelium and oxidation, that is, Ox-LDL formation.[10,21] Cholesterol is a solid fat. It is both taken with food and made in the body. The amount of cholesterol and triglycerides in the blood is significant for assessing the risk of cardiovascular disease. Cholesterol accumulates in the vessels more than the body needs, triggers oxidative stress, and causes vascular elasticity and blockages, leading to hypertension, cardiovascular diseases, infarction, stroke, and paralysis.[22]

In our study model, LDL tg and cholesterol rates in the H2-administered group were significantly lower than in the healthy control group (P<0.05). We believe that H2 increases the protein content in body cells, reduces oxidative stress, reduces lipid oxidative stress, and reduces TG, TK, and LDL ratios, and is effective in preventing the formation of many ischemic vascular damages and protecting tissues and organs.

High-density lipoprotein (HDL) is a particle that has antithrombotic, anti-inflammatory, and anti-atherogenic effects. It also has known antioxidant activity. It prevents the formation of high amounts of cholesterol in the blood by carrying cholesterol from tissues and vessels to the liver. High amounts of cholesterol in the blood cause damage to blood vessels. HDL is called good cholesterol because it removes excess cholesterol from the blood.[23]

Ethical approval

The approval for the study was obtained from the Local Ethics Committee for Experimental Animals of Kafkas University with the ethical committee number 2023/154.

Authors contribution

MK, ÖFB, TD, DA: Research, planning, article scanning, writing-original draft & review.

Conflict of interest

There are no conflicts of interest associated with this research publication, according to the authors.

Data availability

Data will be made available on request

Acknowledgments

None

Funding statement

None

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