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The Effect of Drinking Cold and Hot Sulfur Spring Water on Certain Physiological and Histological Characteristics of Male Albino Rats

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Abstract

Water is essential for all living organisms; however, the use of sulfur spring water for drinking or bathing has historically been complex and associated with certain risks. Therefore, this study aimed to investigate the effects of hot and cold spring waters on the physiological and histological characteristics of male rats. The experiment involved three groups: the first received standard drinking water; the second was treated with hot sulfur spring water; and the third was treated with cold spring water. Treatments were administered at three time points: the start of the experiment [zero time], after 20 days [middle time], and after 40 days [last time]. The results showed no statistically significant differences in the concentrations of glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) among the study groups (control, Cold water, and hot water) at the three time points (zero-time, middle time, and last time) ($P \leq 0.05$). However, the current study revealed a significant increase ($P \leq 0.05$) in GPT and GOT activity in the hot sulfur spring water group, alongside a significant decrease ($P \leq 0.05$) in the cold sulfur spring water group across all assessed time points. Histological examination of the liver revealed congestion in the central veins and necrosis in the hot sulfur spring water group, whereas these effects were not observed in the cold-water group.

Keywords

Sulfur Spring Water;
Water Temperature;
GPT and GOT;
Liver Histopathology;
Hepatotoxicity;
Male Rats

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1.Introduction:

Water is a necessary component for all living things since it is crucial for preserving the internal equilibrium of living tissues, controlling body temperature, moving nutrients, and getting rid of waste products from metabolism. Because of its biological and medicinal qualities, natural mineral and thermal waters have attracted a lot of scientific attention. Natural mineral waters also include a variety of dissolved inorganic components that might affect physiological processes [1].

Hydrogen sulfide (H2S) has become an endogenous regulator of several physiological processes throughout the past 20 years. H2S is a member of the gasotransmitter class of molecules, which also includes carbon monoxide (CO) and nitric oxide (NO). In a variety of cells and tissues, three enzymes are known to be endogenous sources of H2S: 3-mercaptopyruvate sulfurtransferase (3-MST), cystathionine γ-lyase (CSE), and cystathionine β-synthase (CBS) [2]. Hideo Kimura produced more experimental proof of H2S synthesis in the brain in 1996 and suggested—possibly for the first time in "earnest"—that H2S may be an endogenous mediator with physiological or pathological regulatory functions [3].

The focus was on the interconversion of sulfur-containing amino acids rather than the production of H2S, which for a long time was not thought to be biologically significant. Nevertheless, some of the biochemical pathways associated with H2S have been thoroughly characterized over many decades [2]. Most published research on H2S and apoptosis, or other types of cell death, has been produced in vitro in a variety of disease models and is not applicable in a physiological setting. More than 600 published publications now address this distinct area of study; the subject has also been examined in the particular context of several disorders [4].

Additionally, H2S has a role in energy metabolism and glucose control [5]. It contributes to overall glucose homeostasis by improving peripheral tissues' absorption of glucose. Additionally, H2S is essential for mitochondrial activity, affecting ATP synthesis and controlling the generation of metabolic energy [6]. It emphasizes its role in preserving metabolic homeostasis [7] by regulating the activity of vital enzymes in glycolysis, gluconeogenesis, and the citric acid cycle [8]. H2S exhibits a concentration-dependent duality in cellular survival and apoptosis. By preventing cellular apoptosis or boosting cellular tolerance to oxidative stress, physiological levels of H2S provide cytoprotection and prolong cellular life [9]. On the other hand, by affecting mitochondrial activity, H2S may cause apoptosis at larger quantities, especially in cancer cells [10]. The cellular antioxidant defense system can be strengthened by H2S's ability to produce glutathione, a potent antioxidant [11]. Additionally, it has the ability to trigger cellular stress response pathways, such as the Nrf2 pathway, which is essential for the defense against the deleterious consequences of oxidative stress [12].

Drinking cold water has been linked to changes in heart rate, peripheral blood flow, cardiac autonomic nervous system activity, and energy expenditure, according to earlier human investigations. Additionally, the temperature of drinking water may change the temperature of the digestive system and impact cardiovascular and metabolic reactions, making it an important physical characteristic that can impact biological reactions to water consumption [13]. Given the water scarcity experienced in various regions of Iraq in recent years, the potential use of sulfur spring water has become a matter of significant interest. Consequently, there is a need to investigate the effects of consuming such spring water—whether hot or cold—on the physiological indicators and histological characteristics of male white rats, specifically regarding liver tissue and function, as well as lung tissue.

2.Material ad methods:

2.1 Experimental Animals and Design:

The experiment used 45 adult male Sprague Dawley rats that weighed between 175 and 185 grams and were between two and three months old. A veterinarian from the University of Mosul's College of Veterinary Medicine's Animal House Unit reared and examined these rats. Considering comparable weights, the animals were split into three groups of fifteen rats each at random. The experiment was designed as a investigation using two independent variables: exposure duration and drinking water type. A total of forty-five male albino rats were divided into three experimental groups at random based on the type of water they drank: hot sulfur spring water, cold sulfur spring water, and control (tap water). Each group consisted of fifteen rats. Five rats were assigned to each time point, and each group was further separated into three equal subgroups based on the sample periods (day 0, day 20, and day 41). Every sample interval involved the euthanasia of the animals, and all measurements were taken from separate animals without repeated measurements.

2.3 Water Samples and Study Area:

Hot water samples were obtained from sulfur springs in the Hammam al-Alil area of the Mosul Governorate in Iraq. Cold water samples were obtained from sulfur springs in the Hammam al-Alil area of the Mosul Governorate, Iraq. Samples of hot and cold sulfur spring water were collected from certain natural sources and stored appropriately until required. The physicochemical properties of the water, including its temperature and sulfur content, were recorded before to administration.

2.4 Biochemical and Hormonal analysis:

Serum samples were separated into two groups for hormonal and biochemical tests after being stored at -4°C until testing.

2.5 Histological Examination:

Each rat's two testes were taken to the Biology Department at Tikrit University's College of Medicine, where they were kept in a 10% formalin solution. After that, they were dried using progressively more alcohol. The samples were kept in paraffin blocks that had been treated with xylene. Hematoxylin and eosin (H&E) staining was applied after serial slices, each five micrometers thick, were cut with a microtome. The stained sections were seen under a light microscope, according to Bancroft and Gamble. [14].

2.6 Statistical Analysis:

The data were statistically analyzed using GraphPad Prism 10.4.1 software through the Two-way ANOVA test method. The numbers were expressed as arithmetic means ± standard deviation, and significant differences were compared using Duncan's multiple range test at a significance level of $P \leq 0.05$ [15].

3.Results:

The results (Figure 1) showed no statistically significant differences in the concentrations of glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) among the study groups (control, Cold water, and hot water) at the three time points (zero time, middle time, and last time) ($P \leq 0.05$). However, the current study revealed a significant increase ($P \leq 0.05$) in GPT and GOT activity in the hot sulfur spring water group, alongside a significant decrease ($P \leq 0.05$) in the cold sulfur spring water group across all assessed time points. Histological examination of the liver revealed congestion in the central veins and necrosis in the hot sulfur spring water group, whereas these effects were not observed in the cold-water group.

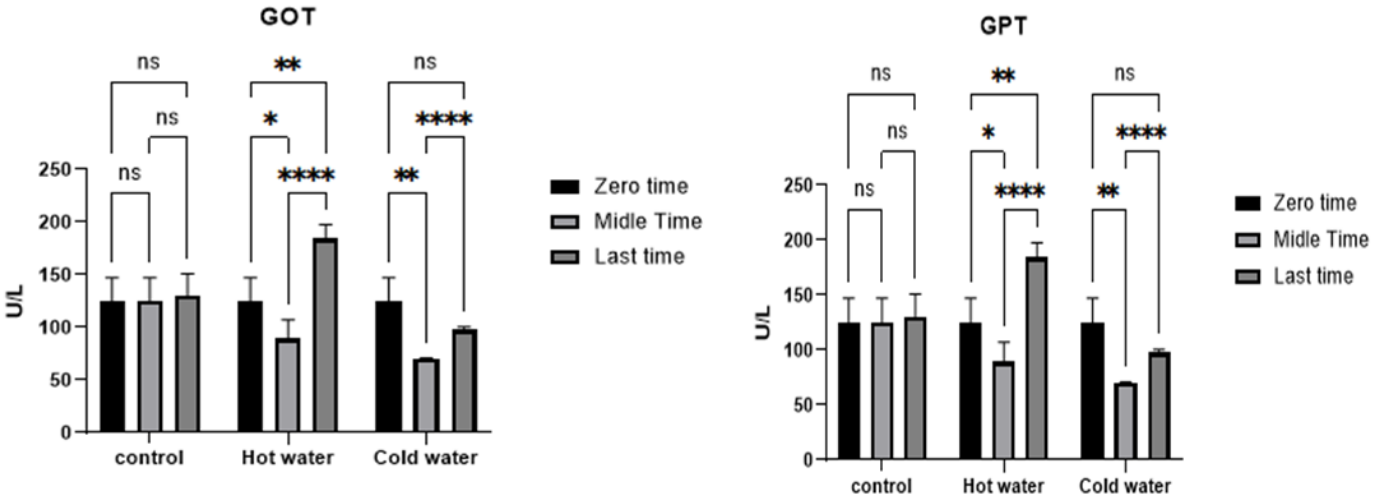

Fig1 Show the effect of hot and cold sulfur spring waters on liver vitality and function in male rats.

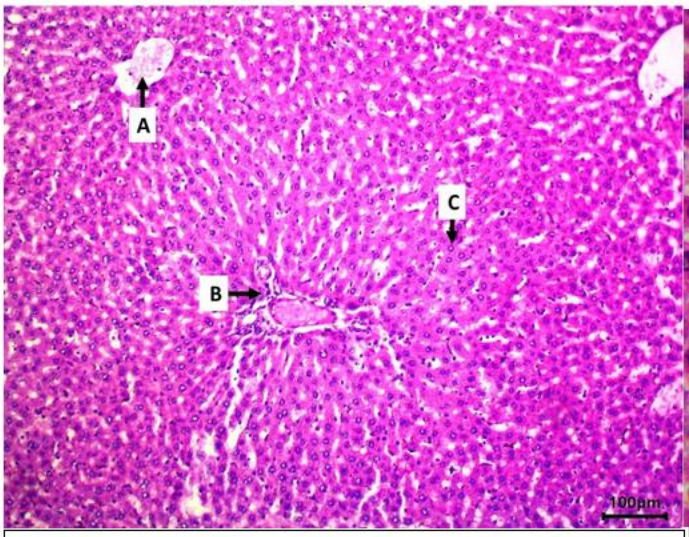

Figure 2: photomicrograph of rat's liver of the control group showing normal architecture of central vein (A), portal area (B) and hepatocytes (C). H&E stain, 100X.

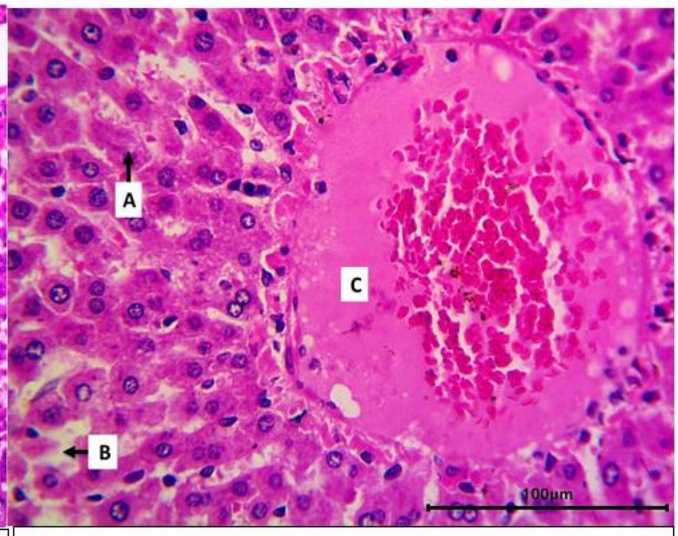

Figure 3: photomicrograph of rat's liver of the Hot treated group showing necrosis of hepatocytes surrounding central vein (A), dilation of sinusoids (C) and recent thrombus in the central vein (D). H&E stain, 400X.

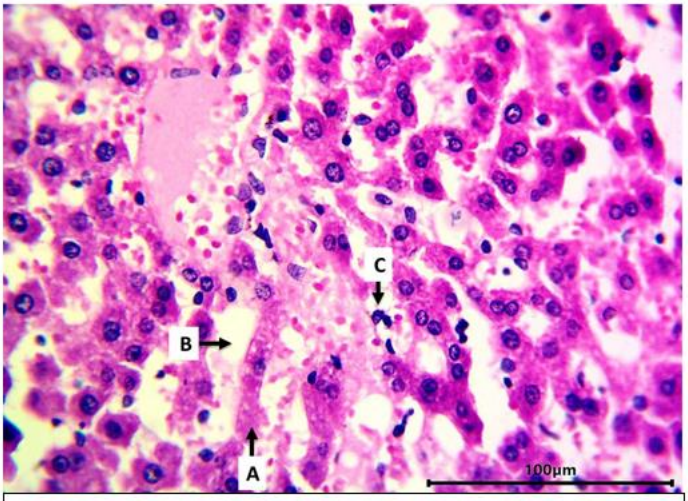

Figure 4: photomicrograph of rat's liver of the Cold treated group showing necrosis of hepatocytes surrounding central vein (A) and portal area (B) with dilation of sinusoids (C) and increased Kuepfer cells (D). H&E stain, 100X.

4. Discussion:

GPT and GOT are key indicators frequently used to measure liver health and function. GPT is more specific to liver cells, whereas GOT is present in numerous tissues—including liver, skeletal muscle, and cardiac muscle cells. In order to detect liver damage or malfunction, they should be assessed together. When these enzyme activities are assessed in conjunction with bilirubin and albumin levels, as well as histological findings, the assessment of liver health becomes more accurate [16]. Previous studies have shown that hepatobiliary biomarkers may vary in sensitivity depending on the type and severity of liver tissue injury [17].as show in fig 1, Examination of rat liver in the heat-treated group (Hot) reveals necrosis (cell death) of hepatocytes surrounding the central vein (A), dilation of hepatic sinusoids (C), and the presence of a recent thrombus within the central vein (D). as show in fig4, Histological sections of a rat kidney in the group showed glomerular atrophy (A), dilation of Bowman's space (B), vacuolar degeneration (C), and necrosis (D) of the epithelial cells lining the renal tubules. as show in fig 3.

The biological effects of sulfur spring water may be attributed to sulfur compounds, particularly hydrogen sulfide (H_2S). Given the long-term nature of the current experiment, high concentrations of H_2S were involved; while H_2S can be toxic at high levels, it also plays a role in regulating oxidative stress, mitochondrial function, inflammation, vascular responses, and cell survival. Since the liver plays a major role in H_2S metabolism, high concentrations can damage hepatic tissue and lead to elevated liver enzymes [18], as indicated by the results of the current study (Figure 1).

The rise in biomarkers observed in the cold-water group at the end of the experiment is highly significant for understanding the importance of water temperature. It suggests that while initial exposure to sulfurous water might cause progressive damage, continued long-term consumption can actually lead to the recovery of liver tissues, as illustrated in Figure 6.

5. Conclusion:

We conclude from the foregoing that sulfur spring waters have both positive and negative effects on liver function and vitality, depending on the duration of exposure and the water temperature. Our current study found that hot sulfur spring water caused hepatic tissue damage and elevated liver enzyme activity; conversely, cold sulfur spring water resulted in less liver tissue damage by the end of the experiment.

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