



Research article

Evaluation of antioxidant status and oxidative stress markers in thermal sulfurous springs residents



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ABSTRACT

Sulfurous springs have been traditionally used in medical treatment for different purposes. These beneficial effects of sulfurous water have been attributed to the presence of sulfurous compounds mainly in the form of hydrogen sulfide (H₂S). The purpose of the present study is to explore the effects of long-term exposure to sulfurous springs on oxidative stress and antioxidant biomarkers responses in individuals who lived nearby the sulfurous springs. The studied area was Al- Hammah sulfurous springs, which is located in the northern part of the Jordan Rift Valley and host many sulfurous springs. Residents in sulfurous springs area are continuously exposed to water and gases emission more than the overall population. We have found that the sulphate levels were 7 times higher in sulfurous springs water samples than control water samples. The majority of the volunteers involved in the present study were more than ten years long residence and lived in range distance between one to five kilometers (less than 3 miles) away from main sulfurous spring, and visited the sulfurous spring at least once a month. We did not find any noticeable symptoms in sulfur spring residents such as headaches, nausea, breathing problems. The total oxidative stress (TOS) and oxidative stress index (OSI) in sulfurous spring residents were lower than control individuals. The total antioxidant capacity (TAC) and total nitric oxide (NO_x) levels were higher in sulfurous spring residents compared to control group. Furthermore, we have highlighted that living nearby the sulfurous springs does not affect oxygen saturation levels (SPO₂) or heart pulse rate. These findings suggest that long-term exposure to sulfurous springs boost the antioxidant capacity and reduce oxidative stress levels in the human body. Hence, visiting sulfurous springs can act as natural remedies to diminish oxidative stress as they show promising potential in several-oxidative stress-related diseases treatment.

1. Introduction

Hydrogen sulfide (H₂S) is one of the main active components present in springs water. The H₂S gas remains trapped within the sulfurous springs and when the water is pumped to the surface H₂S gas is released. The importance of H₂S gas is currently attracting the attention of researchers due to its prospect therapeutic properties [1]. Sulfurous water exerts beneficial anti-inflammatory, keratoplastic, and antipruritic effects [2]. In addition, sulfurous mineral water has been proven for its bactericidal and antifungal properties [3]. H₂S have antioxidant and cell-signaling functions in the central nervous system [4]. The main therapeutic act of sulfurous water is related to the sulfur keratolytic and

has been found to be absorbed through the skin causing vasodilation, analgesia, immune response inhibition, and skin desquamation reduction *via* keratolytic effects [5, 6]. H₂S gas in nature is produced by sulfur bacterial breakdown of organic matter in two ways, either aerobically oxidizing to elemental sulfur then finally transformed to sulfates, or anaerobically reducing sulfates [7]. Human produces a small amount of H₂S gas endogenously in their body's through specific enzymes, including cystathionine-γ-lyase (CSE), cystathionine β-synthase (CBS), and 3-mercapto pyruvate sulfurtransferase (3-MST) from L-cysteine [8].

Oxidative stress can be identified as an imbalance between the equilibrium of prooxidants and antioxidants; favor on the prooxidants induces oxidative damage [9]. The presence of a low amount of reactive

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oxygen species (ROS) can be beneficial, but excess amount are harmful and can cause oxidative damage to biomolecules, leading to many chronic diseases [10]. The human system constructs endogenous antioxidant defense machinery such as antioxidants or enzymes to protect cells from damage due to the accumulation of ROS [10, 11, 12]. The antioxidant defense status would give useful information for health status [13]. Maintaining the endogenous redox balance by H₂S is important to protect human body from oxidative stress [8, 14]. The endogenous H₂S has been reported to act as a vasodilator, angiogenic, anti-apoptotic, anti-inflammatory factor and many others [8, 15]. Human is normally exposed to low H₂S level in their daily life [8]. The toxic effects of H₂S are characteristically dose-related and most notably involve the nervous, cardiovascular, and respiratory systems [16]. Human exposure to low concentrations of H₂S and other sulfur gases emission has been reported to cause headaches, nausea, and other symptoms [17, 18], short-term exposures to high levels of H₂S may cause eye damage [19], while acute inhalation of H₂S induces breathing problems [20]. Furthermore, long-term exposure of H₂S at the workplace raises the mean cell hemoglobin derivative methemoglobin and sulfhaemoglobin levels [21].

In our present study, we aim to investigate the effect of long-term exposure of sulfurous springs on oxidative stress and antioxidant balance in individuals living nearby sulfurous sulfur springs. The studied area was Al-Hammah district, which is located in the northern part of the Jordan Rift Valley (Irbid Governorate, Jordan), and hosts more than 20 hot sulfurous springs that contain elevated levels of H₂S in their surroundings.

2. Materials and methods

2.1. Water and volunteers blood samples measurements

Six water samples from different water sources in Al-Hammah sulfurous springs area were collected in July 2018. The chemical properties of the water samples were tested using ion chromatography (IC) workstation (Thermo Fisher Scientific, MA, USA). All water samples were filtered through a 0.45 μm filter before running through the IC, and known standard concentrations were used to estimate the concentrations of chemicals in water samples (Thermo Fisher Scientific, MA, USA).

All volunteers were apparently healthy individuals. We further excluded those who have blood pressure, diabetes, any chronic disease or taking any medications. Blood samples were collected from 250 volunteers aged between 18 and 45 years, those volunteers were 39 % males and 61 % females, and lived permanently nearby Al-Hammah sulfurous springs for the last 5 years. The control group samples were collected from 250 volunteers living in Der-Allah region, which has similar mean sea level (MSL) and geographic features, but without sulfurous springs exposure. The blood samples from all volunteers were collected between July 2018 to December 2018. Serum was obtained by placing 5 ml of the whole blood in an empty tube without any anticoagulant and allow the blood to clot for 30 min, then blood samples were centrifuged at 2500 rpm for 15 min, finally the serum was collected by pipette and transferred into a new tube. The present study was approved by the Institutional Review Board (IRB) Committee (2017–111/36) at Jordan University of Science and Technology. At all times, ethics was respected for the investigation, the anonymity of the subjects that participated was kept confidential, and informed consent was obtained from all participants.

2.2. Measurements of blood oxygen saturation (SpO₂) and pulse rate

Oxygen level (oxygen saturation) of the blood and pulse rate was measured using compact fingertip transmissive pulse oximetry, upon

arrival; all the volunteers were seated in a room maintained at 24 ± 1 °C for a minimum of 10 min to ensure hemodynamic stabilization, and then pulse oximetry reading was recorded.

2.3. Analysis of blood samples

Total oxidative stress and total antioxidant capacity were measured in plasma using an OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit according to the manufacturer's instructions (Cell Biolabs Inc. CA, USA). The assay is calibrated with hydrogen peroxide (H₂O₂) and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H₂O₂ Eq/L). The total nitric oxide (NO_x) levels were measured using the Griess assay (Promega Inc. WI, USA).

Calculation of Oxidative Stress Index (OSI): The OSI was defined as the ratio of the TOS to TAC level. Specifically, OSI (arbitrary unit) = TOS (μmol H₂O₂ Eq/L)/TAS (μmol uric acid Eq/L) [22, 23].

2.4. Statistics analysis

The results are expressed as mean ± SD. Statistical analysis was performed using an SPSS software package, version 22.0 (SPSS, Inc. IL, USA). Statistical comparisons were conducted using the two-tailed Student's t-test. *P* < 0.05 was considered significant.

3. Results

3.1. Data collection and sampling

To evaluate the sulfurous springs conditions and contents the temperature, pH, total dissolved solids (TDS), electrical conductivity (EC), sulphate and nitrate levels were detected using a calibrated instrument as shown in Table 1. The temperature and pH were measured in the field during the time of sample collection. The sulfurous spring main sources temperature was ranged between (57 °C to 59 °C), and the PH (7.42 to 7.72) in the three main springs locations. The sulphate levels were about 7 times higher than tap water and groundwater, whereas the sulphate levels for all three sulfurous spring were very close to each other. We did not notice any differences in nitrate levels, TDS and EC between sulfurous springs samples.

To investigate the effect of long-term exposure to the sulfurous spring environment, a total of 250 volunteers living in Al-Hammah district were randomly selected. Informed consent was obtained from all subjects who participated in the present study. Around 62% of sulfurous spring residents involved in the study visited the main sulfurous spring water source at least once a month, whereas, 37 % visited the spring main source more than once a week and 1 % visited the spring main source once a week as shown in Fig. 1 A. We did not find any noticeable symptoms in sulfur spring residents such as headaches, nausea, breathing problems. The living duration and residence distance from the Al-Hammah sulfurous spring area were considered during the present study and shown in Fig. 1 B and C. The majority of the volunteers involved in the present study (83.9 %) were living in the sulfurous spring area for more than ten years, while 14.64 % were living in sulfurous spring area between five to ten years, and only 1.46 % of the individuals were living in the sulfurous springs area for at least one year but less than five years.

Furthermore, the majority of the residents volunteers involved in our present study were living nearby the sulfurous springs area in a range distance between one to five kilometers (less than 3 miles) away from the Al-Hammah sulfurous springs. About 67.77% of residents were living close to the main sulfurous springs between one to five kilometers, 26.38 % of the residents were living between five to ten kilometers, and only 5.85 % were living in a distance more than ten kilometers away from sulfurous springs main water source (Fig. 1C).

Table 1

Temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), nitrate and sulphate measurements of the studied sulfurous springs and other different water sources.

Sample	Source	Temperature	pH	EC micro s/cm	TDS mg/l	Nitrate ppm	Sulphate ppm
1	Sulfurous spring (location A)	59 C°	7.72	1023	704	1.45	106.1
2	Sulfurous spring (location B)	57 C°	7.41	1020	699	1.56	107.1
3	Sulfurous spring (location C)	58 C°	7.71	1022	701	1.41	108.9
4	Groundwater well	17 C°	7.98	132	87	1.71	15.8
5	Tap water (city water)	20 C°	7.68	977	666	1.74	16
6	Tap water (city water)	18 C°	7.75	975	663	1.68	16.7

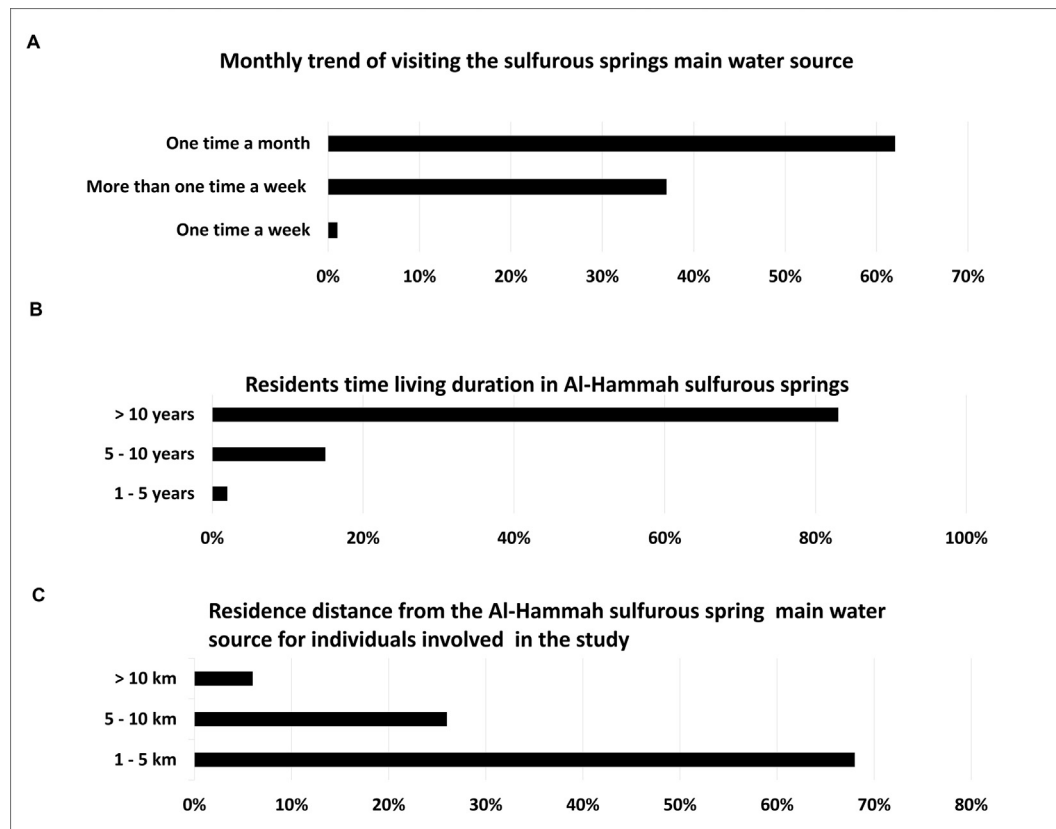


Fig. 1. The monthly trend of visiting sulfurous springs main water source, living duration and residence distance from the Al-Hammah sulfurous springs. (A) shows the monthly trend of visiting Al-Hammah sulfurous springs (B) shows that the majority of sulfurous springs main water source residents (83.9 %) were living in the sulfurous springs area for more than ten years, while residence distance chart (C) shows that the majority of sulfurous spring residents (67.77%) were living close to the main sulfurous springs between one to five kilometers.

Table 2

Evaluation of oxygen saturation and pulse rate by pulse oximetry values in control and sulfurous springs residents.

Sample	Control	Sulfurous springs residents	P-value
O ₂ Content %	96.75 ± 0.2	96.89 ± 0.2	0.67
Pulse Rate (beat/min)	88.35 ± 1.08	88.14 ± 1.18	0.88

3.2. Measurements of blood oxygen saturation (SpO₂) and pulse rate

Pulse rate and oxygen saturation (SpO₂) using pulse oximetry were measured in both control and sulfurous springs residents as shown in Table 2. Results showed that oxygen saturation levels in control individuals were 96.75% ± 0.2, compared to 96.89% ± 0.2 in sulfurous springs residents. Oxygen saturation levels were found to be within normal ranges (95–100 %), and no significant difference were found between the two studied groups. The mean pulse rate in the control and sulfurous springs residents were 88.35 ± 1.08, and 88.14 ± 1.18 beats/min respectively. Both rates were found to be within the normal pulse

rate for healthy adults (normally, 60 to 100 beats/min).

3.3. Analysis of the samples: measurements of total oxidative stress (TOS), antioxidant capacity (TAC), oxidative stress index (OSI), and total nitric oxide (NO_x) levels

In order to determine if the sulfurous springs long time exposure affect the antioxidant/oxidative stress markers, TOS, NO_x, TAC and OSI, were evaluated in the serum from sulfurous springs residents and control individuals.

Serum TOS was significantly elevated in control individuals (8.90 ± 0.70 mmol of H₂O₂/L) compared to the sulfurous springs residents (3.90 ± 0.04 mmol of H₂O₂/L) as shown in Table 3. The levels of the total antioxidant capacity were significantly elevated in the sulfurous spring residents (0.68 ± 0.06 mM UAE) compared to the control group (0.48 ± 0.06 mM UAE) as shown in Table 3. In the control individuals, the OSI was significantly elevated (p < 0.001), compared to the sulfurous springs residence. Total nitric oxide was significantly elevated in the sulfurous

Table 3

Total oxidative stress (TOS), total antioxidant capacity (TAC) and oxidative stress index (OSI) and total nitric oxide (NO_x) in control and sulfurous springs residents. The data shows that the TOS was significantly elevated ($P < 0.01$) in control individuals (8.9 ± 0.7 mmol of H₂O₂/L) compared to the sulfurous springs residents (3.9 ± 0.04 mmol of H₂O₂/L), the TAC shows a highly significant difference ($P < 0.05$) between control and sulfurous springs residents as it was higher in the sulfurous springs residents (0.68 ± 0.06 mM UAE) than in the control group (0.48 ± 0.06 mM UAE). The levels of NO_x were significantly elevated ($P < 0.05$) in the sulfurous springs residents (3.91 ± 0.30 μM) compared to the control group (2.66 ± 0.09 μM).

Sample	Control	Sulfurous spring residents	P-value
Total oxidative stress (TOS) mmol equivalent of H ₂ O ₂ /L	8.9 ± 0.70	3.9 ± 0.04	<0.001
Total antioxidant capacity (TAC) mM equivalent of uric acid/L	0.48 ± 0.06	0.68 ± 0.06	0.03
Oxidative Stress Index (OSI) (arbitrary unit)	34.86 ± 6.54	9.20 ± 1.99	<0.001
Total nitric oxide (NO _x) (μM)	2.66 ± 0.09	3.91 ± 0.30	<0.05

The statistically significant difference is found when ($p < 0.05$), the \pm a value are the standard error of the mean.

The OSI was defined as the ratio percentage of the TOS level to TAS level, an indicator of the degree of oxidative stress. Specifically, OSI (arbitrary unit).

springs residents (3.91 ± 0.30 μM) compared to the control group (2.66 ± 0.09 μM) as shown in Table 3.

4. Discussion and conclusion

H₂S emission from different sources has become a serious human health and ecological safety issue. This study investigated the effect of long-term exposure to sulfurous springs gases emission on oxidative stress and antioxidant capacity. The studied area was Al-Hammah that hosts many hot sulfurous thermal springs and recognized as medicinal water in the northern part of Jordan (Irbid governorate). According to 2018 Jordanian census, a total of 20324 individuals (9296 females and 11028 males) are living in that area. The serum levels of NO_x and TAC were higher and ROS levels were lower in sulfurous springs residents compared to control individuals, thus indicating that sulfurous water has a useful activity against ROS accumulation and boosting the antioxidant response. The OSI index was employed to emphasize the differences in oxidative stress and antioxidant values between the sulfurous springs residence and the control group. In particular, for those H₂S emission known to cause a decrease in oxidative stress and an increase in antioxidants, we have found that OSI index is decreased in sulfurous springs residence compared to the control group.

Moreover, TAC in the serum of the sulfurous springs residents was higher compared to the control group. The observed antioxidant effects in the sulfurous springs residents could be partially interpreted by the possible action of H₂S gas in scavenging the endogenous free radicals [24] or upregulates the transcription of antioxidant genes [25]. TAC should be considered with oxidative stress status because the measurement reflects outcomes in a dynamic system. The elevated antioxidant capacity sometimes may not necessarily be a desirable situation if it reflects a response to increased oxidative stress. Similarly, a decrease antioxidant profile may not necessarily be an undesirable situation if the measurement reflects decreased production of ROS [26].

ROS-induced oxidative stress is extensively used to monitor the biological effects, it is also important to increase the antioxidant capacity of biological fluids, cells, and extracts [12]. Increased ROS concentration reduces the amount of bioactive NO by chemical inactivation to form toxic peroxynitrite (ONOO⁻) which can scavenge NO [27]. Peroxynitrite can "uncouple" endothelial NO synthase enzyme to become a dysfunctional superoxide-generating enzyme that contributes to vascular oxidative stress [28]. On the other hand, NO released from NOS enzymes

have been known for its antioxidant properties [28]. Measurement of the total antioxidant capacity in control individuals provides an indication of the overall capability to counteract ROS. We have found that control individuals have lower antioxidant capacity compared to sulfurous springs residents, thus control individuals relatively have higher ROS levels. Furthermore, NO_x is affected by ROS, due to the fact that ROS can induce nitric oxide synthases (NOS) uncoupling and thus reduce the NO_x levels.

Decreased ROS accumulation in individuals living near the sulfurous springs is likely explained by H₂S-scavenger effect from the H₂S gas emitted from sulfurous springs. One previous study has shown that H₂S has the capacity to affect oxidative processes in ventilator-induced lung injury in a rodent model and its underlying molecular signaling pathways remain elusive [29]. This study has shown that ventilation with moderate tidal volumes of 12 mL/kg for 6 h led to an excessive formation of ROS in mice lungs which was prevented by supplemental inhalation of 80 parts per million of H₂S [29].

Different sulfurous springs around the globe have various physico-chemical characteristics depending on their compositions, ions, and amounts of salts, but the common denominator is the presence of the sulfide species such as H₂S, hydrosulfide ion (HS⁻) and sulfide anion (S²⁻) [30]. H₂S was primarily considered a toxic environmental pollutant at high concentrations [16]. The beneficial effect of sulfurous spring water has been reported for it is medical usage in traditional medicine [31]. Several studies have focused on the negative impact of H₂S exposure from sewers or workplaces. However, the constant exposure to low environmental levels of H₂S has not been fully investigated. We postulated that sulfide-rich environment in the sulfurous springs surrounding could affect the blood oxygen saturation and heart pulse rate; since high sulfide species released in the air, might binds to ferric heme in the metalloproteins (e.g., hemoglobin) and prevents oxygen binding. Usually, when H₂S is inhaled, it will be absorbed into the bloodstream, and then detoxified primarily by oxygen bound to hemoglobin, and in the process, it is oxidized into a sulfate or thiosulfate that is excreted through the kidneys. Trace amounts of the H₂S gas that are unoxidized are eliminated by the lungs as dissolved H₂S. This detoxification process takes place very rapidly, and therefore H₂S does not act as a cumulative toxin in the human body [32]. However, in certain circumstances when the dose of the H₂S inhaled exceeds the detoxification capacity, it can then impair mitochondrial respiration by inhibiting the activity of the cytochrome c oxidase enzyme, thus reducing overall energy production. However, we did not find any noticeable effect of H₂S on oxygen saturation and heart pulse rate, probably due to the present of molecular adaptation mechanism, and endogenous H₂S physiological concentration not toxic concentration. A study has reported a few cases with acute H₂S intoxication caused by inhalation of H₂S evaporated from the water of a thermal spring due to using thermal water from illegal artesian well directly through the centralized network [33]. Thus, the use of unprocessed thermal water through the individual artesian directly is believed to be the cause of the accumulation of H₂S. The concentrations of sulfide and thiosulfate in blood samples from victims were at least 14 and 7 times higher than the levels in healthy persons [33].

Finally, it is important to highlight that there is still a lack of studies that determine more precisely the biological effects arising from the H₂S gas emission among sulfurous springs, which could entail a more efficient use in the clinical practice. Given that Jordan is one of the countries with the greatest number of sulfurous springs reserves in the world, there is great potential for appreciation and investigation of the biological characteristics of Jordanian sulfurous springs. In summary, our study shows that living near Al-Hammah sulfurous springs did not induce any noticeable symptoms such as headaches, nausea, breathing problems or any effect on oxygen saturation and heart pulse rate. Furthermore, living nearby Al-Hammah sulfurous springs improves the antioxidant capacity and efficiently reduces oxidative stress in human due to H₂S gas exposure. Thus, visiting sulfurous springs can help in reducing oxidative stress and boost overall health/antioxidant capability.

Declarations

Author contribution statement

Zaid Altaany, Almuthanna Alkaraki, Ahmed Abu-siniyeh: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Waleed Al Momani, Omar Taani: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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