Sodium dithionate (Na$_2$S$_2$O$_4$) induces oxidative damage in mice mitochondria heart tissue

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**Abstract**

Sodium dithionate (Na$_2$S$_2$O$_4$) is an inorganic sodium salt, a bleaching agent that has a reducing agent role and is generally used as food additive or preservative in the production of diverse foods and beverages. The aim of this study is to determine the effects of Na$_2$S$_2$O$_4$ on cardiac mitochondria damage and biochemical parameters in male mice. Na$_2$S$_2$O$_4$ were administered orally at doses of 10, 20, 50 and 100 mg/kg to male mice for 45 days. Heart mitochondria were isolated for the evaluation of oxidative stress biomarkers such as mitochondrial function, reactive oxygen species (ROS), lipid peroxidation (LPO), protein carbonyl (PC) content, catalase activity (CAT) and glutathione content (GSH). Blood samples were collected and Creatine phosphokinase (CPK), Creatine kinase-MB (CK-MB) and Troponin I were quantified in the serum. Mitochondrial function was significantly (P < 0.001) decreased and oxidative stress biomarkers including, ROS, LPO and PC were significantly enhanced. Also, CAT activity and GSH content were significantly decreased by Na$_2$S$_2$O$_4$-treated groups in heart mitochondria when compared to the control group. Na$_2$S$_2$O$_4$ administration elevated the serum levels of CPK, CK-MB, and troponin I (especially, at high doses) compared with the control group. These findings suggest that Na$_2$S$_2$O$_4$ induces mitochondria toxicity and cardiac damage especially at high doses with a dose-dependent manner.

1. Introduction

Sodium dithionate (Na$_2$S$_2$O$_4$) is a white crystalline, an inorganic sodium salt, a bleaching agent that has a reducing agent role and is generally used as food additive or preservative in the production of diverse foods and beverages [1]. Na$_2$S$_2$O$_4$ can potentially reduce residual oxide, eliminate excess dye, and unintentional pigments [2]. Although it was reported that the Na$_2$S$_2$O$_4$ and other sulfite salts are generally nontoxic, arguments arose regarding its application which led to extensive regulations [3].

The mammalian heart is an organ with obligate aerobic. A continuous supply of oxygen is crucial for cardiac viability and its function. The imbalance between oxygen demand and supply can damage the heart tissue and cause necrosis. Under anaerobic situations, mammalian heart muscle cannot produce sufficient energy to preserve necessary cellular procedures; thus, a constant oxygen supply is vital to sustain heart function and viability [4]. Hypoxia is a prominent pathological feature of heart disease that results in deformity and malfunction of the heart tissue [5].

Mitochondrial integrity is fundamental for cell function and cell life. Mitochondrial function disturbances lead to cell function disruption and cell death. Moreover, mitochondria need to constant flow of oxygen for proper functioning. Mitochondrial organelle of heart cells are involved in numerous cellular processes beyond its role in energy production [6]. The prominence of this organelle for heart tissue homeostasis has been extensively studied whose disorder can lead to cell death and heart failure [7].

Oxygen is the main determining factor of myocardial function, viability and gene expression. It is also essential in the production of reactive oxygen species (ROS), which can engage in cell signaling processes or can produce cellular damage and death [7].

It was demonstrated that Na$_2$S$_2$O$_4$ induced toxicity in cells in different organs through oxygen deficiency which can lead to pathological features in cardiovascular disease [8–10]. Na$_2$S$_2$O$_4$ helps the formation and release of various ions in the body, and sulfur dioxide (SO2) which creates free radicals and can cause hypoxia which is
 responsible for numerous disorders, including organ toxicity and damage to heart tissue and its function [9,11,12]. Cardiac toxicity caused by increased oxidative stress can generate ischemic heart disease (IHD) [13, 14]. After the intake of Na2S2O4, the larger number of blood metabolic factors can attack to macromolecules in cells and induce peroxidation of the lipids, protein oxidation and DNA damage [15,16]. In cell stress condition, excessive accumulation of mitochondrial calcium can trigger mitochondrial protein translocation (MPT) dysfunction and mitochondrial toxicity. This abruptly increases the permeability of the mitochondrial inner membrane, and the opening of non-selective pores in the inner membrane allowing molecules of smaller than 1.5 kDa to pass through [15,17]. When the "permeability barrier" in the cell mitochondrial is disrupted, low-weight molecular compounds pass freely through the membrane while larger proteins are barred, which forms pressure-induced colloid osmosis and subsequently mitochondrial swelling [18]. However, indefinite opening of the pores (which usually occurs during a pathological complication) causes structural alterations in mitochondria, expansion of the inner membrane and mechanical disruption of the outer membrane, resulting in other consequences for mitochondria during MPT [19]. These include weakening of the membrane and detachment of oxidative phosphorylation (OXPHOS), inhibition of respiration due to loss of common agents or cytochrome C, and higher oxidative stress [17,18,20]. Also, mitochondrial membrane depolarization leads to the reversal of ATP synthase activity, during which, higher oxidative stress [17,18,20] . Also, mitochondrial membrane depolarization leads to the reversal of ATP synthase activity, during which, higher oxidative stress [17,18,20].

Mitochondria during MPT [19]. These include weakening of the membrane and detachment of oxidative phosphorylation (OXPHOS), inhibition of respiration due to loss of common agents or cytochrome C, and higher oxidative stress [17,18,20]. Also, mitochondrial membrane depolarization leads to the reversal of ATP synthase activity, during which, higher oxidative stress [17,18,20].

In an attempt to compensate for the lost trans-mitochondrial potential, ATP is hydrolyzed rather than produced [21]. Na2S2O4 is widely used in industrial breads, sugar products (rock candies) and caning without sufficient monitoring and management. The use of these chemical additives in various food products seems to be a serious threat to human health. Thus, the present study investigated the potential effect of different doses of sodium dithionate on induced cardotoxicity, mitochondrial heart damage and biochemical parameters of toxicity in male mice.

2. Materials and method

2.1. Chemicals

The sodium dithionate (purity ≥ 87 %) used in this study were provided from Merck (Darmstadt, Germany) and Ketamine hydrochloride (purity ≥ 99 ), Xylazine hydrochloride (purity ≥ 99 %), 3-[4, 5-dimethylthiazol-2-yl]–2, 5-diphenyltetrazolium bromide (MTT; purity ≥ 98 %), 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA; purity ≥ 97 %), 2, 4-dinitrophenylhydrazine (DNPH; purity ≥ 97 %), thiobarbituric acid (TBA; purity ≥ 98 %), Trichloroacetic acid (TCA; purity ≥ 98 %) and 5, 5-Dithiobis[2-nitrobenzoic acid] (purity ≥ 98 %) used in this study were provided from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animal study

40 Male albino mice weighing 25 ± 5 g were used in this study. Animals were divided into five groups (8 mice in each) and housed in chambers at 25 °C for 12 h in a dark and light cycle for free access to food and water. All animal tests were achieved in accordance with the approvals on animal experiments by the ethics committee of the Mazandaran University of Medical Sciences (MAZUMS) with the ethic number: IR.MAZUMS.REC.98.4897. Na2S2O4 was orally administered to mice at doses of 10, 20, 50 and 100 mg/kg for 45 days. The normal saline group was considered as control group.

2.3. Blood samples and heart mitochondria preparation

After 24 h of last gavage, animals were anesthetized by Ketamine-Xylazine (K, 100 mg/kg; X, 10 mg/kg) and blood samples were taken by cardiac puncture and the serum was removed for biochemical experiments. Hearts were brought out and washed with cold buffer at 4 °C, and then were fractionated with scissors and were homogenized. Heart mitochondria were isolated under different centrifugation conditions. Oxidative stress biomarkers in mitochondria were investigated. Isolated mitochondria were dispersed in tris buffer for the measurement of mitochondrial function and oxidative damage including glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PC) content through catalase (CAT) activity tests. For the evaluation of reactive oxygen species (ROS), isolated mitochondria was scattered in breathing buffer [22]. All experiments were performed in triplicate.

2.4. Biochemical assays and analysis

To examine the heart damage, vital enzyme activities such as Creatine phosphokinase (CPK), Creatine kinase-myoglobin binding (CK-MB) and Troponin I were assessed using commercial kit (Pars-Azmoon, Iran).

2.5. Measurement of protein concentration

Protein content of each sample was assessed in isolated mitochondria of heart according to Bradford method. Bovine serum albumin was used as a standard. Briefly, samples were incubated with Coomassie blue and after 10 min, absorbance was determined at 595 nm by spectrophotometer (UV-1601 PC, Shimadzu, Japan) [23].

2.6. Measurement of mitochondrial function (MITT assay)

MTT evaluation protocol was employed for mitochondrial viability. Samples (0.5 mg/ml of mitochondria) were incubated with MTT for 30 min. Formazan produced by mitochondria was assessed by ELISA reader (Tecan, Rainbow Thermo, Austria) at 570 nm [22].

2.7. ROS mitochondrial determination

ROS levels were measured using the DCFH-DA reagent. Briefly, 10 μl of DCFH-DA was added to 1000 μl of sample and stored at 4 °C for 15 min. Then, by a fluorimeter, the absorbance was measured at the excitation wavelength of 480 nm and the emission wavelength of 520 nm [24].

2.8. Mitochondrial PC content measurement

Determination of protein carbonyl content was performed using DNPH reagent. A total of 80 μl of trichloroacetic acid (20 % w/v) was added to 100 μl of each sample and incubated at 4 °C for 15 min. Samples were centrifuged at 6500g for 10 min, and the supernatant liquid was discarded. The bottom sediment was thoroughly dispersed in 500 μl of 0.1 M, NaOH. Then, 500 μl of 10 mM DNPH was dissolved in 2 M HCl and was added to the samples. A blank sample was also prepared by adding 500 μl of 2 M HCl (DNPH-free) to the protein sample, incubated for half an hour in a dark condition. Then, 500 μl of trichloroacetic acid (20 % w/v) was added to each of them. Precipitated protein was collected after centrifugation and finally the absorbance was read at 365 nm with an absorption coefficient of 22,000 M⁻¹ cm⁻¹ [24].

2.9. Mitochondrial LPO measurement

The lipid oxidation was measured by the TBA reagent. Briefly, 0.2 ml of mitochondrial suspension was added to 0.1 ml of TBA reagent including 0.5 % normal HCl, 15 % TCA and 0.3 % TBA and were thoroughly mixed and incubated in the boiling water bath for 30 min. After cooling, 0.2 ml of n-butanol was added and the mixture was shaken vigorously, and then centrifuged at 3500g for 10 min. The supernatant (n-butanol layer) was separated and the absorbance was measured at 532 nm by ELISA reader (Tecan, Rainbow Thermo, Austria) [25].
2.10. Mitochondrial GSH content measurement

For GSH content determination, 0.25 ml of 20 % trichloroacetic acid was added to 1 ml of mitochondrial fraction. The mixture was centrifuged at 1000g for 20 min. Subsequently, 1 ml of the clear supernatant solution was taken and 2 ml of 0.3 M sodium hydrogen phosphate and 0.5 ml of 0.4 % DTNB were added to each sample, which was then incubated for 15 min. The absorbance was measured at 412 nm. According to the standard curve of glutathione, sample concentration was calculated and expressed as μM [26].

2.11. Measurement of CAT activity in mitochondria

A total of 20 μl of the mitochondrial fraction was poured into a test tube using tris buffer. Then, 4800 μM of reaction medium including sodium phosphate buffer (50 mM, pH 7.0) and H2O2 (10 mM) was added to it. Finally, the absorbance was red at the 240 wavelength nm [26].

2.12. Statistical analysis

The results were reported as mean ± SD. Statistical analyses were done by the GraphPad prism software, version 6. One-way analysis of variance (ANOVA) followed by the post-hoc Tukey test was used to compare the means. The P < 0.05 were considered as minimal level of significance.

3. Results

3.1. Effects of different doses of Na₂S₂O₄ on the oxidative stress biomarkers

The results of the present study indicated that the mitochondrial function (MTT assay) significantly (P < 0.001) were reduced by all administered doses of Na₂S₂O₄ in mice heart tissues when compared to the control (CT) group. Mitochondrial function decreased in all doses of Na₂S₂O₄ treated groups (10, 20, 50 and 100 mg/kg; 73.62 %, 76.17 % and 79.81 %; respectively) when compared with the control group (Fig. 1).

ROS formation in heart mitochondria showed that this marker of oxidative damage significantly increased (p < 0.01) after the administration of Na₂S₂O₄ doses (50, 100 mg/kg) groups compared to the control group. Na₂S₂O₄ treatment at the dose of 100 mg/kg caused a significant increase (P < 0.05) of ROS level in heart mitochondria compared to the Na₂S₂O₄ doses (20 mg/kg) group (Fig. 2).

The levels of the malondialdehyde (MDA) as lipid peroxidation product were measured in this study. The results indicated that the MDA levels significantly increased (P < 0.001) only at the dose of 100 mg/kg in heart mitochondria after the administration of Na₂S₂O₄ when compared with the control and Na₂S₂O₄ (10, 20, 50 mg/kg) groups (Fig. 3).

Protein carbonyl levels were significantly elevated (P < 0.01 and P < 0.001) by Na₂S₂O₄ at doses of 50 and 100 mg/kg as compared to the control group. Also, a significant increase (P < 0.01 and P < 0.001) in protein carbonyl content by Na₂S₂O₄ (50 and 100 mg/kg) was observed when compared with Na₂S₂O₄ (10 mg/kg) group (Fig. 4).

As shown in Fig. 5, the catalase (CAT) activity significantly decreased (P < 0.001) in Na₂S₂O₄ (20, 50, 100 mg/kg) treated groups when compared to the control group. CAT activity in the mice heart mitochondria significantly reduced by Na₂S₂O₄ administration at the dose of 20, 50, 100 mg/kg as compared to Na₂S₂O₄ (10 mg/kg) group (Fig. 5).

Contents of GSH were determined in mice heart mitochondria which was significantly produced (p < 0.001) in all treated groups of Na₂S₂O₄ at all doses (10, 20, 50 and 100 mg/kg) as compared to the control group. Mitochondrial GSH content significantly (P < 0.001) decreased in Na₂S₂O₄ (50, 100 mg/kg) groups when compared with the Na₂S₂O₄ (10 mg/kg) group. This biomarker was significantly (P < 0.001) lower in Na₂S₂O₄ (100 mg/kg) group compared to the Na₂S₂O₄ (50 and 100 mg/kg) groups (Fig. 6).

3.2. Effects of Na₂S₂O₄ on serum biochemical parameters in treated mice

For the determination of the heart damage induced by Na₂S₂O₄, the activity of the cardiac damage biomarkers including CPK, CK-MB and troponin I were investigated in this study. The results of the present study indicated that the levels of the serum CPK were significantly (P < 0.001) elevated in the Na₂S₂O₄ (20, 50 and 100 mg/kg) groups compared to the control and Na₂S₂O₄ (10 mg/kg) groups (Table 1).

In this study, the level of CK-MB, as indicator of cardiac toxicity, significantly increased in the Na₂S₂O₄ (100 mg/kg) group when compared to the control and Na₂S₂O₄ (10, 20 and 50 mg/kg) groups.
As shown in Table 1, Troponin I, as a factor of cardiac injury, became significantly higher ($P < 0.01$) in the $\text{Na}_2\text{S}_2\text{O}_4$ (20 and 100 mg/kg) groups compared to the control and $\text{Na}_2\text{S}_2\text{O}_4$ (10 mg/kg) groups (Table 1).

### 4. Discussion

In this study, we found that sodium dithionate has induced oxidative stress in heart mitochondria and increased cardiac damage biomarkers in male mice. These pathological effects were produced dose-dependently.

Sulfating agents or sulfites, also named as sodium hydrogen sulfite are used as food additives in large amounts and abundance in the food industry, cosmetics and chemical industries. To the best of our knowledge, there is very little researches that have been done on the effects of $\text{Na}_2\text{S}_2\text{O}_4$ on mitochondrial in animals, so there is very little data in this area. Using high quantities of sodium dithionate in food industries can cause hazard problems. Based on the CODEX 2001, the sulfur compounds residual maximum permissible level (MPL) in white sugar is 15 mg/kg of body weight per day. Also, the maximum permissible dose of $\text{Na}_2\text{S}_2\text{O}_4$ is recommended 10 ppm in bread (Institute of Standards & Industrial Research of Iran (ISIRI)) [27].

The heart uses several metabolic fuels such as amino acids, ketones, lactate, glucose and fatty acids. Mitochondria are involved in all the above processes provide the heart with enough energy. During oxygen deficiency, there is a reduction in the amount of energy which is insufficient for cardiac necessities. The imbalance between oxygen demand and supply can induce heart tissue damage and necrosis[4]. As a prominent pathological feature of heart disease, hypoxia causes the deformity and malfunction of the heart tissue [5]. Oxygen is also essential element for the production of reactive oxygen species (ROS), which is involved in cell signaling processes or can produce cellular
ROS can be generated in the heart cardiomyocytes via different mechanisms such as mitochondria oxidative phosphorylation as a product of metabolism in normal cellular aerobic. Therefore, the main manner from which the heart originates enough energy can also cause in the generation of ROS [7,28].

In the present study, mitochondrial function in the mice heart was shown to have reduced after Na$_2$S$_2$O$_4$ administration compared to the control group. All doses of Na$_2$S$_2$O$_4$ decreased the mitochondrial function but the highest diminishment was observed at the dose of 100 mg/kg (Fig. 1). Mitochondria has a central role in cellular homeostasis, energy production, ROS generation and cell death. These processes can cumulatively occur via numerous stressing conditions, amongst which hypoxia is the most prominent. It was stated that Na$_2$S$_2$O$_4$ can induce toxicity in cells via oxygen deficiency that leads to mitochondria dysfunction [4-8,10]. Approximately, 98 % of oxygen is consumed by mitochondria. Therefore, hypoxia induce mitochondrial dysfunction that leads to alterations in the functioning of various tissues such as cardiac muscles. In this case, Na$_2$S$_2$O$_4$ can cause mitochondrial dysfunction in heart tissue via generation of hypoxia as observed in our study (Fig. 1).

Membrane proteins, phospholipids and DNAs can be oxidized by ROS including hydroxyl radicals (OH) and superoxide anions (O$_2^-$) that are implicated in an extensive range of pathological conditions. On the other hand, their significance has been confirmed in restricted sub-classes of cardiac diseases and cardiac toxicity [29]. Given that Na$_2$S$_2$O$_4$ causes mitochondrial damage, we found significantly extended formama damage and death [7]. ROS can be generated in the heart cardiac tissues and we evaluated the activities of CAT and SOD in heart mitochondria of different Na$_2$S$_2$O$_4$ (50 mg/kg) groups.

Table 1

| Parameter          | Control (Mean ± SD) | Na$_2$S$_2$O$_4$ (10 mg/kg) (Mean ± SD) | Groups Na$_2$S$_2$O$_4$ (20 mg/kg) (Mean ± SD) | Na$_2$S$_2$O$_4$ (50 mg/kg) (Mean ± SD) | Na$_2$S$_2$O$_4$ (100 mg/kg) (Mean ± SD) |
|--------------------|---------------------|------------------------------------------|-----------------------------------------------|----------------------------------------|-----------------------------------------|
| CPK                | 58.33 ± 17.62       | 76.67 ± 9.292                           | 328.7 ± 26.73 *** ††††                       | 271.0 ± 81.54 ** †††††                 | 396.3 ± 80.62 *** ††††††                |
| CK-MB              | 87.67 ± 9.292       | 98.00 ± 2.000                           | 116.7 ± 10.02                                | 135.3 ± 17.01                         | 285.0 ± 69.74 ** ††††††††             |
| Troponin I         | 3.284 ± 1.585       | 6.527 ± 1.000                           | 29.40 ± 7.892 ** †††††††††                   | 16.35 ± 3.302                         | 29.96 ± 10.16 ** †††††††             |

* (p < 0.05), ** (p < 0.01), *** (p < 0.001): Significantly different from control group (CT).
# (p < 0.05), ## (p < 0.01), ### (p < 0.001): significantly different from Na$_2$S$_2$O$_4$ (10 mg/kg) group.
$$ (p < 0.001):$$ significantly different from Na$_2$S$_2$O$_4$ (20 mg/kg) group.
## (p < 0.01): significantly different from Na$_2$S$_2$O$_4$ (50 mg/kg) group.
SD, standard deviation; CPK, creatine phosphokinase; CK-MB, creatine kinase-myoglobin binding.

In accordance with this, Lin et al. [34] showed that Na$_2$S$_2$O$_4$ caused a decline in the C6 glial cells GSH, while the level of LPO was prominently elevated. It was stated that the cells GSH content is expended to neutralize the ROS accumulated in hypoxia condition [34]. In the current study, we treated the mice with different doses of Na$_2$S$_2$O$_4$ and we evaluated the activities of CAT and GSH antioxidant enzymes in the heart mitochondria for assessing the antioxidant defense system. Significant reduction in CAT activity and GSH content by Na$_2$S$_2$O$_4$ proves that the CAT and GSH are consumed in the cell to scavenge the free radicals created in mitochondria as a defensive response mechanism (Figs. 5 and 6).

Na$_2$S$_2$O$_4$ can exacerbate the damage to cellular macromolecules such as membrane lipids and proteins. Structural linkage between Na$_2$S$_2$O$_4$ in muscles reduces oxygen uptake by the absorption of water; it also releases toxic sulfur dioxide (SO$_2$) compounds into mitochondrial heart muscle causing the disruption of mitochondrial respiratory cycle, and ADP to not convert to ATP, which eventually stops the production of energy [37].

Another hypothetical mechanism for Na$_2$S$_2$O$_4$ to enhance the oxidative damage in heart mitochondria is the possible release of the SO$_2$ from Na$_2$S$_2$O$_4$ into the blood circulation. It was stated that myocardium mitochondrion is the most sensitive organelle exposed to the SO$_2$. The mitochondrial dysfunction of cardiac muscles after...
exposure to SO₂ has been reported. Moreover, mitochondrial membrane potential (MMP), mitochondrial DNA contents, ATP contents, and the oxidase activity of cytochrome c were diminished after SO₂ exposure [38]. In this study we found that Na₂S₂O₄ (10–100 mg/kg) via oral administration for 45 days induced mitochondrial dysfunction and myocardium damage. According to the results of our study, oral administration of Na₂S₂O₄ stimulates cardiotoxicity through worsening the tissue damage, and increasing serum biochemical markers of myocardial damage such as CPK, CK-MB and troponin I levels (Table 1).

CPK is an enzyme that is essentially found in the heart and skeletal muscles. A CPK test is performed to detect muscular dystrophy and elevated level of CPK indicates the damage of the muscle or muscle disease. It was demonstrated that high levels of CPK can be due to enzyme leakage from the muscle cells [39]. It was stated that during heart attack, CPK leaks out from the heart alongside other enzymes [40].

In this study, the elevated CPK might be due to cardiac muscle damage that is created by induced hypoxia in heart muscles via Na₂S₂O₄ administration. Creatine kinase (CK) and its MB isoenzyme (CK-MB) are the most generally used serologic experiments and has been the gold standard method for the diagnosis of myocardial injury prior to the extensive adoption of troponin [41]. In our research, levels of CK-MB and troponin I (heart damage biomarkers) were increased by Na₂S₂O₄ (especially at high doses) after 45 days of treatment. Alterations of these biomarkers may depend on time exposure and the dose of Na₂S₂O₄. Elevated CK-MB was reported in patients with pulmonary emboli, myocardial damage after fetal hypoxia and ischemia reperfusion injury in heart of animals essentially due to hypoxia [42–44]. Troponin I which is one of the best cardiac biomarkers and a surrogate marker for the diagnosis of myocardial injury was enhanced in mice treated by sub-chronic administration of Na₂S₂O₄ (Table 1). Similarly, previous studies have shown that induced hypoxia and/or hypoxic-ischemia could elevate cardiac troponin I in human and animals [45–48].

We used of 10, 20, 50 and 100 mg/kg of Na₂S₂O₄ in this study. Although these doses seems high for this compound, but, these selected doses is because of different metabolism rate and different biokinetics of species (animals and humans). It was revealed that smaller animals, such as mice have a much faster metabolic rate than humans. Therefore, the content of mitochondrial in muscle cell of a mouse would be enhanced than that of a human to produce more energy from one feeding [49]. Also, the basal metabolic rate per gram of body weight is seven times greater in mice than in humans [50,51]. The administered dose or the length of the feeding period can relatively describe this difference in magnitude of the effect between humans and mice, but metabolic differences rate might play a major role. Related gastrointestinal absorption possibly will be obtained when doses in humans (kg body weight) are 5–7 times lower than in rats. It was stated that the clearance of small molecules is more rapid in the mouse and rat and is related to the rate of circulation, where hepatic and renal blood flow is 7 times lower than in rats [52]. Also, the basal metabolic rate per gram of body weight is seven times greater in mice than in humans [53]. Previous study showed that Na₂S₂O₄ at concentration of 5 mg/ml could significantly decrease the mitotic indexes (MI), increase the frequency of chromosomal aberrations CA, sister chromatid exchanges (SCEs) and abnormal cell in human lymphocytes. Also, higher concentration of Na₂S₂O₄ (5 and 10 mg/ml) can induce High chromotid and chromosome breaks [54]. Although the doses used in this study are higher than maximum-permitted level (MPL) of sulfites (≥10 mg/kg) in consumed foods recommended by Codex Alimentarius Commission (CAC), European Union (EU), but noteworthy point is that the various species have diverse in content of mitochondria in ranging between 22.0 % and 37.0 %. Also, there is a close association between the mitochondrial volume density, heat rate and the basal oxygen consumption rate in any group of animals [55].

In general, it can be assumed that Na₂S₂O₄ diminished the mitochondrial function, increased the levels of oxidative stress biomarkers and biochemical parameters in heart mitochondria of mice by increasing the dosage and exposure time. These heart pathologic effects can be due to the hypoxia-induce mechanism of Na₂S₂O₄.

5. Conclusion

In summary, with reference to all the obtained data, Na₂S₂O₄ has induced cardiotoxicity in a dose-dependent manner by enhancing the ROS, LPO, PC content and biochemical (CPK, CK-MB and Troponin I) levels, reduction of GSH content and CAT activity and diminishing mitochondrial function via induced hypoxia in heart tissue cells. Although The maximum permitted levels of the sulfites (10 mg/kg) in different kinds of food has been recommended and were stabilized by the CAC, EU and Food Standards Australia New Zealand (FSANZ) and other countries, but further studies should be focused on the deleterious effects of chemicals such as Na₂S₂O₄ that are used in various foods.

Declaration of Competing Interest

The authors declared no conflicts of interest.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Roozbeh Zare gashti and Hamidreza Mohammadi. The first draft of the manuscript was written by Roozbeh Zare gashti. Hamidreza Mohammadi read and approved the final manuscript.

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