Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2025, 38(2)

Determination of the Effective Dose of Sodium Thiosulfate in Adult Rats Treated with Nicotine

Zena Shakir Al-Tamemi[1*] and Baraa Najim Al-Okaily[2]
[1,2]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

Received: 4 July 2023 Accepted: 30 August 2023 Published: 20 April 2025
doi.org/10.30526/38.2.3645

Abstract

Sodium thiosulfate (STS) is a possible therapeutic candidate molecule in a range of diseases and drug-induced toxicities due to its antioxidant, anti-inflammatory, and anti-apoptotic capabilities. The current study aimed to find the effective dose of STS in adult male rats given nicotine by evaluating serum MDA and blood partial pressure of oxygen (PO_2) levels. Thirty-six adult male Wistar rats, *Rattus norvegicus* (weighing 190–220 g) with an age of 3–3.5 months, were chosen randomly and separated into six equal groups, administered for 28 days. Animals in the control group were administered an intraperitoneal (i.p.) injection of normal saline, while those in groups 1, 2, 3, 4, and 5 received repeated doses of an i.p. nicotine injection of 1.5 mg/kg b.wt. One hour later, they were I.P. injected with 150, 250, 350, 450, and 550 mg/kg b.wt. of STS, respectively. Both medications continued to be given for 28 days. At the end of the experiment, serum malondialdehyde (MDA) and PO2 were measured. Results showed a negative relationship between blood PO_2 concentration and five successive doses of sodium thiosulfate, but a positive linear relationship among successive doses of sodium thiosulfate on serum MDA concentration was observed. The estimated amount of sodium thiosulfate that caused a considerable decrease in serum MDA levels and a rise in blood PO_2 was found to be 450 mg/kg bw.t. The current study's findings show that different doses of sodium thiosulfate can significantly reduce the harmful effects of nicotine exposure by reducing the formation of oxidative stress and the detrimental effects on respiratory function, which are characterized by an increase in blood PO_2 levels.

Keywords: Sodium thiosulfate, Effective dose, MDA, Blood, Partial pressure, Oxygen.

1. Introduction

Thiosulfate, or sodium thiosulfate ($Na_2S_2O_3$, or STS), is an oxidative component of hydrogen sulfide (H_2S), the third family member, and an intrinsic signaling chemical. Acute cyanide toxicity, cisplatin toxicity during cancer treatment, and calciphylaxis in dialysis patients are

currently all treated clinically with STS. STS is a possible therapeutic candidate molecule with antioxidant and anti-inflammatory capabilities that is able to target numerous biological mechanisms underlying diverse illnesses and drug-stimulated toxicities. (1). To scavenge nitric oxide, superoxide, and hydroxyl radicals, various dosages of STS were used. Thiosulfate's ability to combat free radicals is thought to be due to either an improvement in the antioxidant system or an innate capacity (2). Sodium thiosulfate, a non-toxic substance approved by the FDA, is used for treating cyanide poisoning and calciphylaxis (3). It acts as an antioxidant, removing reactive oxygen species (ROS) (4) and activating antioxidant enzymes (5). It also has vasodilatory, antiapoptotic, and anti-inflammatory properties (6). Lipid peroxide, also known as MDA, is linked to cancer and can harm enzymes and DNA (7). Smoking is a major airborne pollutant affecting human lung health, causing the development of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (8). These pollutants can damage biomolecules, initiate pathogenic cellular processes, and contribute to lung illnesses (9). Despite lung antioxidant defenses, ongoing exposure to nicotine leads to persistent free radical production (10). Nicotine exposure to rats has been linked to a decrease in tight junction protein (TJP) gene expression, which is crucial for maintaining healthy lungs. This is due to an increase in oxidative stress-associated genes (11), which can lead to inflammation, changes in histone acetylation, and oxidation of lipids, proteins, and DNA. This can lead to respiratory disorders such as asthma, chronic pulmonary obstructive disorder, cystic fibrosis, acute respiratory distress syndrome, pneumonitis, lung cancer, and obstructive sleep apnea (12, 13). Studies suggest that cigarette smoke exposure may result in oxidative stress or degradation of vital biological macromolecules, potentially leading to the deactivation of the protein 1-protease inhibitor. (14, 15, 16). Studies show that nicotine injection can cause lung complications in mice, including alveolar damage, emphysema, blood vessel congestion, hemorrhage, edema, lung fibrosis, and lymphocyte infiltration (17). These effects vary depending on the nicotine injection period. Long-term nicotine usage activates the first enzyme in catecholamine production, tyrosine hydroxylase, and interferes with antioxidant defense mechanisms. Additionally, nicotine stimulates the production and release of hormones like norepinephrine and epinephrine (18). Smoking negatively impacts pulmonary functions, leading to obstructive and restrictive respiratory issues. Postoperatively, smokers experience a decline in blood gas measures and a higher incidence of pulmonary problems (19). Smoking during pregnancy can lead to poor perinatal outcomes (20). The production of hydroxyl radicals by bound tar radicals can damage DNA. However, the diversity of test variations makes it difficult to widely accept MDA as a biomarker for oxidative damage to lipids. Nicotine also increases serum pro-inflammatory cytokines like IL-6 and MDA in rats (21). Thus, the objective of the current investigation was to assess, through the use of one respiratory system-related metric, the optimal dose of STS that can modify the oxidative stress brought on by nicotine in the lung.

2. Materials and Methods

2.1. Animals and experimental design

Thirty-six male Wister rats (*Rattus norvegicus)* and weighing 190–220 g., 3-3.5-month-old, were used in the current study. Animals were kept in cages with a 12-hour light/dark cycle at 22–25°C. Animals had full access to water and pellets throughout the study period. Rats were randomly assigned into six equal groups (6 rat per treatment) and experimented for up to 28 days

after acclimatization for 15 days. The rats in the control group received i.e. injections of normal saline 0.1 ml/100 gm b.wt. once daily for 28 days; those in the test groups (G) 1, 2, 3, 4, and 5 received 1.5 mg/kg b.wt. i.p./day injections of nicotine (Nicotine 72290 >97%, KP 243-248o, Liquid 162.24 Switzerland), then, after an hour, they received i.p. injections of STS (20268 K05 purity 99.50, SDFCL, SD Fine Chemical Limited, Mumbai, India) doses 150, 250, 350, 450, and 550 mg/kg b.wt, respectively, also for 28 days. Blood samples were collected at 14 and 28 days to quantify the serum malondialdehyde (MDA) content using a modified method. As shown by (23). The MDA assay kit was obtained from Solarbio Life Science. Operation Equipment: Spectrophotometer.

2.2. Determination procedure

1. Preheat the spectrophotometer for more than 30 minutes, set zero with distilled water.
2. Add reagents in **Table 1:**

Table 1. The reagents used.

Reagent (µL)	Test tube (T)	Blank tube (B)
MDA working reagent	600	600
Sample	200	-
Distilled water	–	200
Reagent lll	200	200

The mixture was incubated at 100 °C for 60 minutes (tightly closed to prevent moisture loss) before cooling in an ice bath. Centrifuge at 10000 g for 10 minutes at room temperature to remove insoluble materials. Take 200 L of supernatant in a 1 mL glass cuvette and measure the absorbance at 450 nm, 532 nm, and 600 nm. A450 =A450(T)-A450(B), A532 =A532(T)-A532(B), A600 =A600(T)-A600(B).

2.3. Calculation

A (nmol/mL)= (6.45)(A532 -A600)-1.29A450)VrvVs = 5(6.45)(A532 -A600)-1.29A450). Arterial blood samples were collected from rats in tubes specific for blood gas analysis (ABG). The blood partial pressure of oxygen (PO_2) was calculated using the OPTI CCA-TS_2 blood gas and electrolyte analyzer (OPTI Medical SN.OP6-005567 USA). Two times of blood sample collection were enough for the determination of the STS effective dose.

2.4. Method of estimating effective dose of sodium thiosulfate

The isolate with the maximum melanin production underwent the following procedures according to (8) to extract and purify melanin.

2.5. Physico-chemical characterization of partially purified melanin pigment

Probit analysis is a method of analyzing the relationship between a dose and a response. Groups of animals are given different doses of a drug. The percent response at each dose level is recorded. These data may then be analyzed using Probit Analysis. The Probit Model assumes that the percent response is related to the log dose as the cumulative normal distribution. That is, the log doses should be used as variables to read the percent response from the cumulative normal, using the normal distribution rather than other probability distributions. Hence, the comparison of different drugs is done using response rates of fifty percent. The ED (effective dose) is expressed mathematically by equation (1):

$$Y = mX + b \quad (1)$$

where ***m*** is the slope and ***b*** is the intercept of the *y*-axis.

2.6. Statistical analysis

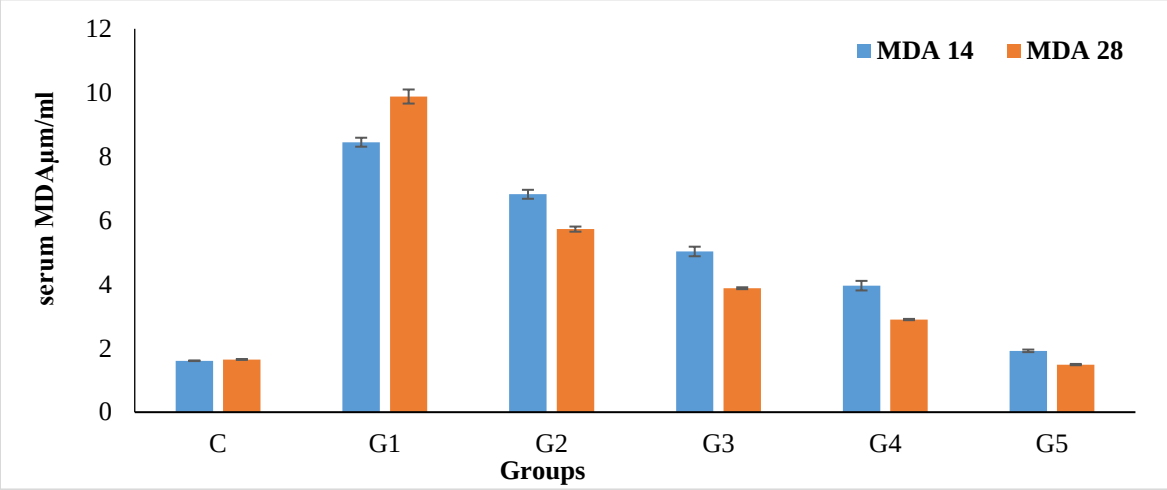
The data (mean±SE) obtained were assessed using two-way analysis of variance (ANOVA) in SPSS (Version 22). To ascertain the level of significance between the various data at the level of $p \leq 0.05$, the LSD test was employed (24).

2.7. Clinical signs

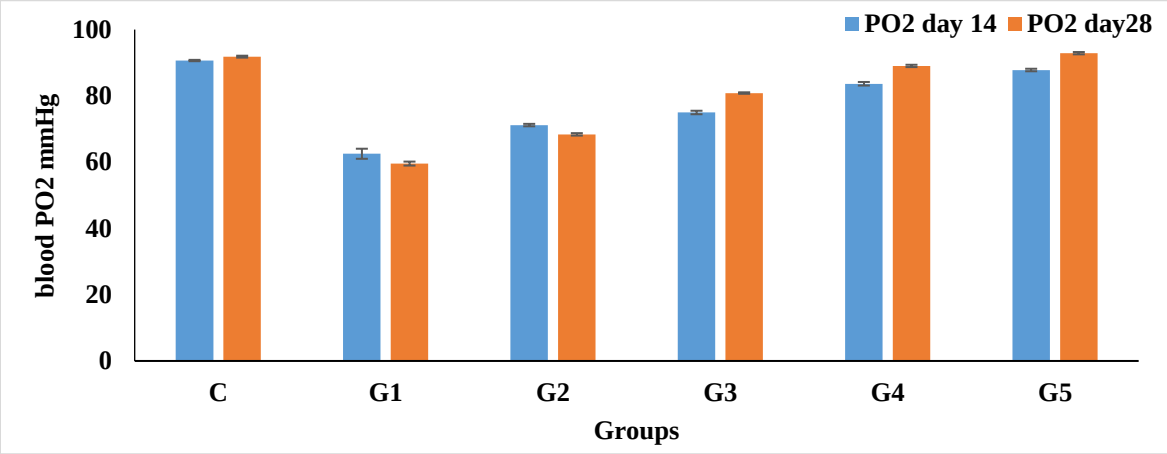
A few minutes after nicotine injection, signs of lack of nervous and muscular coordination were noticed. Signs became more clear after a few minutes of nicotine ip injection, including aggressiveness, convulsions, and severe muscular spasms, particularly in the abdominal muscles, as well as the tail, neck, and head flaring upwards.

3. Results and Discussion

Results showed a significant ($P \leq 0.05$) decrease in blood PO_2 for all treated groups as compared with control group **Figure 1-B**. Current results showed a significant ($P \leq 0.05$) increase in this parameter for the G1 group that receives a lower dose of STS with 1.5 mg/kg of nicotine as compared with control, G2, G3, G4, and G5. It was also noticed that there was a gradual, significant increase in oxygen level in the blood simultaneously with the increase of STS as an antioxidant dose for day 14. Whereas the results of day 28 showed a significant ($P \leq 0.05$) decrease in the G1 group as compared with all other groups, while there were no significant ($P \geq 0.05$) differences between the C and G5 groups, and there was a significant ($P \leq 0.05$) increase in these groups as compared with other groups for the same period. As a comparison within groups in day 14 and day 28, results showed that there was a significant ($P \leq 0.05$) increase in G3, G4, and G5 groups for day 28 as compared with groups in 14 days, while the control group showed no significant ($P \geq 0.05$) differences between the two periods, and the G1, G2 groups showed a significant ($P \leq 0.05$) decrease in day 28 as compared with day 14. Results indicate that PO_2 level increased gradually with an increase STS dose. These results showed that the effect of stress induced by nicotine was gradually modulated by the successive doses of sodium thiosulfate and was more effective in 28 days than its effect after 14 days. Results showed a significant ($P \leq 0.05$) decrease in blood PO_2 for all treated groups as compared with control group **Figure 1-B**, current results showed a significant ($P \leq 0.05$) increase in this parameter for G1 group that receive lower dose of STS with 1.5 mg/kg of nicotine as compared with control, G2, G3 G4 and G5 it was also noticed that there was a gradual significant increase in oxygen level in blood simultaneously with the increase of STS as an antioxidant dose for day 14. whereas the results of day 28 showing a significant ($P \leq 0.05$) decrease in G1 group as compared with all other groups while there was no significant($P \geq 0.05$) differences between C and G5 groups and there was a significant ($P \leq 0.05$) increase of these groups as compared with other groups for the same period. as a comparison within groups in day 14 and day 28, results showed that there was a significant ($P \leq 0.05$) increase in G3, G4, G5 groups for day 28 as compared with groups in 14 day while control group which showed no significant ($P \geq 0.05$) differences between the two periods and G1, G2 groups were showed a significant ($P \leq 0.05$) decrease in day 28 as compared with day 14. Results indicate that PO_2 level increased gradually with increase STS dose. These results showed that the effect of stress induced by nicotine was gradually modulated by the successive doses of sodium thiosulfate was more effective in 28 days than its effect after 14 days.



A



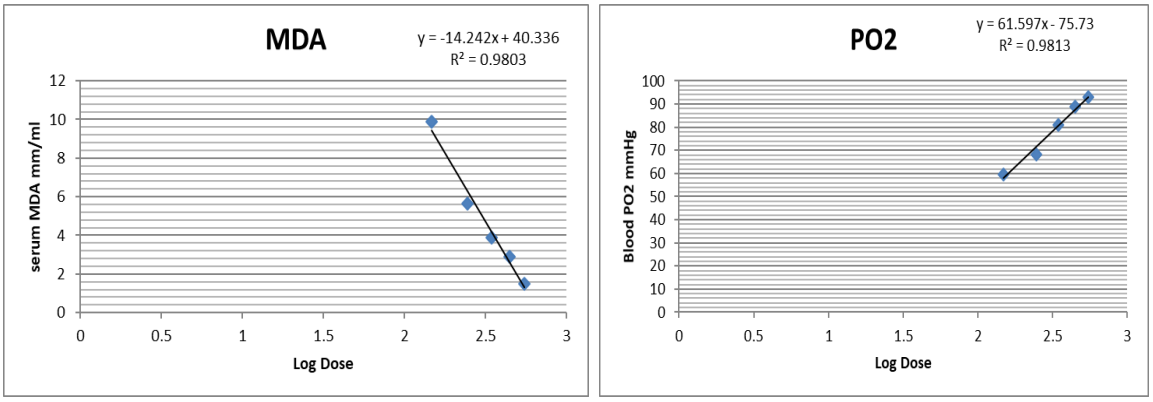
B

Figure 1. Effect of successive doses of STSon (A) serum malondialdehyde MDA and (B) blood PO2 concentrations in adult male rats with nicotine after 14, 28 days. C=control group were injected with normal saline. G1= Treatment group were injected ip with nicotine 1.5 mg /kg b.wt.+ ip injected with 150 mg/kg b.wt of sodium thiosulfate . G2 = Treatment group was injected ip with nicotine 1.5 mg /kg b.wt+ ip injected with 250 mg/kg b.wt.W of sodium thiosulfate . G3= Treatment group were injected ip with nicotine 1.5 mg /kg b.wt+ ip injected with 350 mg/kg b.wt of sodium thiosulfate. G4= Treatment group were injected ip with nicotine 1.5 mg /kg b.wt+ ip injected with 450 mg/kg b.wt of sodium thiosulfate .G5= Treatment group were injected ip with nicotine 1.5 mg /kg b.wt+ ip injected with 550 mg/kg b.w of sodium thiosulfate. Significant differences between groups are indicated by different letters. The results presented as the mean ± SE; N = 6 rats; ($P \leq 0.05$)

3.1. Determination of the effective dose of sodium thiosulfate

Depending on the results shown in **Figures 1A** and **1B**, a maximally significant change (suppression or elevation) in the parameters was recorded after four weeks of sodium thiosulfate treatment. Accordingly, obtained results at week four were used to estimate the ED of STS, where serum concentrations of MDA and blood PO_2 were used as response parameters. A positive linear relationship was observed between successive doses of sodium thiosulfate and serum MDA concentration (**Figure 2A**) from the equation of the straight line. The ED of STS caused a marked decrease ($P \leq 0.05$) in MDA concentration. Whereas, **Figure 2B** showed negative relationships between blood PO_2 concentration and five successive doses of sodium thiosulfate. ED of STS caused marked increases ($P \leq 0.05$) in blood PO_2 concentration. The coefficients of determination were equal to 0.9803 and 0.9813 for MDA and PO_2, respectively. From the equation, the estimated ED of sodium thiosulfate that causes a marked depression in

serum MDA and an elevation in blood PO_2 concentrations was found to be equal to 450 mg/kg B.W.



A **B**

Figure 2. The impact of various STS dosages on adult male rats' (n = 6) blood PO_2 and serum MDA concentrations after 28 days.

Results of the present experiment showed that sodium thiosulfate acts as an antioxidant against the i.p. injection of nicotine (1.5 mg/kg b.w.) to induce oxidative stress in adult male rats. A decrease in serum MDA concentration characterized the results with an increased dose of sodium thiosulfate. Nicotine is the primary alkaloid in tobacco and a highly addictive chemical found in cigarettes. Nicotine has been linked to an increased risk of liver failure, cardiovascular disease, type 2 diabetes, pulmonary problems, various malignancies, and infertility. Furthermore, it is easily absorbed through the skin and respiratory tract. Nicotine can also reach bloodstream peak levels. Notably, the cytochrome P450 2A6 (CYP2A6) enzyme in the human liver plays a critical role in nicotine metabolism, converting nearly 80% of the absorbed nicotine into cotinine. (25). Additionally, many studies reported that nicotine reduced the level of antioxidants superoxide dismutase (SOD) and glutathione (GSH) in rat lung tissue (26, 27, 28).. Current results revealed a significant increase in blood PO_2 with increasing the dose of STS, indicating its role in alleviating the harmful effects of nicotine. Other studies have shown that H_2S is a key signaling molecule produced in low levels by mammalian cells (29). It has a variety of effects on various body organ systems, such as the nervous system (both central and peripheral), cardiovascular, gastrointestinal, and respiratory systems, and has the capacity to control cellular metabolism. Also, liposomal encapsulated STS improved mitochondrial metabolism profile, capillary tube production, and wound healing (30). Endogenous hydrogen sulfide (H_2S) in mitochondria produces a metabolite called thiosulfate; it has been employed as an antioxidant to eliminate ROS, diminish them, and stimulate gene expression of antioxidant enzymes. The cardioprotective advantages of STS against reperfusion injury (IR) to the heart and congestive heart failure (CHF) are also thought to include vasodilatory, antiapoptotic, and anti-inflammatory actions by keeping the mitochondria active, which interacts with caspase enzymes, scavenges free radicals, chelates calcium ions, and increases ventricular H_2S production (6). Subsequent lipid peroxidation may be related to lung disease. Considering that the level of MDA in the study of lung cancer patients is significantly higher (31),. Other studies demonstrated the use of various doses of STS and evaluated the safety and tolerability of the H_2S donation sodium thiosulfate (STS) in patients with acute coronary syndrome (ACS). Patients undergoing coronary

angiography for ACS were given STS via an intravenous soon after arrival at the catheterizing laboratory with fixed dosage endpoints (0, 2.5, 5, 10, 12.5, and 15 grams) (32). Other studies' results have been obtained using intravenous STS. It has also been applied directly to cutaneous lesions in doses of 250 mg/mL, with the lesions clearing up after 6 weeks. These treatments' success is thought to be multifaceted. STS is well-known for its anti-calcification, vasodilatory, and antioxidant effects (33). The doses of STS in this study were adopted taking into consideration the lower and upper limits of the doses used in other studies, and they are within the permissible limits and do not conflict with doses in other studies. Doses in other studies were varied from one another; the aim of the present study was to determine the effective dose of STS as an ameliorating antioxidant that can reduce the harmful effect of nicotine on the respiratory system by measuring PO_2 in blood and serum MDA.

4. Conclusion

The current study's findings show that different doses of sodium thiosulfate can significantly reduce the harmful effects of nicotine exposure by reducing the formation of oxidative stress and reducing the detrimental effects on respiratory functions, which are characterized by an increase in blood oxygen levels PO_2. The effective dose of STS was estimated to be 450 mg/kg b.w. based on concentrations of various indicators, including MDA and PO_2, measured across 14 and 28 days.

Acknowledgment

I am grateful to all of those with whom I have had the pleasure to work during this study, especially my supervisor, who provided me with extensive personal and professional guidance and taught me a great deal about both scientific research and life in general.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

No funding.

Ethical Clearance

Animal care and treatment in this study was carried out at the College of Veterinary Medicine within the University of Baghdad in strict accordance with the code of ethics for animal experiments, and ethical approval was given through the local committee of animal care and use (P.G. 899, date 27-4-2023).

References

1. Zhang MY, Dugbartey GJ, Juriasingani S, Sener A. Hydrogen sulfide metabolite, sodium thiosulfate: Clinical applications and underlying molecular mechanisms. Int J Mol Sci. 2021;22(12):6452. https://doi.org/10.3390/ijms22126452.
2. Ravindran S, Boovarahan SR, Shanmugam K, Vedarathinam RC, Kurian GA. Sodium thiosulfate preconditioning ameliorates ischemia/reperfusion injury in rat hearts via reduction of oxidative stress and apoptosis. Cardiovasc Drugs Ther. 2017;31(5):511–24. https://doi.org/10.1007/s10557-017-6743-4.

3. Yang X, Liu Y, Xie X, Shi W, Si J, Li X. Use of the optimized sodium thiosulfate regimen for the treatment of calciphylaxis in Chinese patients. Ren Fail. 2022;44(1):914–22. https://doi.org/10.1080/0886022X.2022.2078462.
4. Grover P, Khanna K, Bhatnagar A, Purkayastha J. *In vivo*-wound healing studies of sodium thiosulfate gel in rats. Biomed Pharmacother. 2021;140:111797. https://doi.org/10.1016/j.biopha.2021.111797
5. Hayden MR, Tyagi SC, Kolb L, Sowers JR, Khanna R. Vascular ossification–calcification in metabolic syndrome, type 2 diabetes mellitus, chronic kidney disease, and calciphylaxis–calcific uremic arteriolopathy: the emerging role of sodium thiosulfate. Cardiovasc Diabetol. 2005;4(1):1–22. https://doi.org/10.1186/1475-2840-4-4.
6. Shekari M, Gortany NK, Khalilzadeh M, Abdollahi A, Ghafari H, Dehpour AR. Cardioprotective effects of sodium thiosulfate against doxorubicin-induced cardiotoxicity in male rats. BMC Pharmacol Toxicol. 2022;23(1):32. https://doi.org/10.1186/s40360-022-00566-z.
7. Ascar IF, Khaleel FM, Hameed AS, Alabboodi MK. Evaluation of Some Antioxidants and Oxidative Stress Tests in Iraqi Lung Cancer Patients. Baghdad Sci J. 2022;19(6 Suppl.):1466. https://doi.org/10.21123/bsj.2022.7597.
8. Alqayim MA. Antiatherogenic effects of Vitamin E against lead acetate induced hyperlipidemia. Mesop Environ J. 2015;1(2):85–95. https://www.iraqoaj.net/iasj/download/4435b6a407082e83.
9. Ito H, Yamashita Y, Tanaka T, Takaki M, Le MN, Yoshida L-M. Cigarette smoke induces endoplasmic reticulum stress and suppresses efferocytosis through the activation of RhoA. Sci Rep. 2020;10(1):12620. https://doi.org/10.1038/s41598-020-69282-3.
10. Thimmulappa RK, Chattopadhyay I, Rajasekaran S. Oxidative stress mechanisms in the pathogenesis of environmental lung diseases. In: Oxidative Stress in Lung Diseases. Springer; 2020. p. 103–37. https://doi.org/10.1007/978-981-32-9366-3_5.
11. Hamady JJ, Al-Okaily BN. Alveolar gene expression of tight junction protein in nicotine rats treated with zinc and vitamin D. Int J Health Sci (Qassim). 2022;6:232–46. https://doi.org/10.53730/ijhs.v6nS9.12218.
12. Maurya PK, Dua K. Role of oxidative stress in pathophysiology of diseases. In: Role Oxidative Stress Pathophysiology Disease; 2020: p. 1–297. https://doi.org/10.1007/978-981-15-1568-2.
13. Nawfal AJ, Al-Okaily BN. Effect of the Sublethal Dose of Lead Acetate on Malondialdehyde, Dopamine, and Neuroglobin Concentrations in Rats. Worlds Vet J. 2022;12(3):311–5. http://dx.doi.org/10.54203/scil.2022.wvj39.
14. Wooten JB, Chouchane S, McGrath TE. Tobacco smoke constituents affecting oxidative stress. In: Cigar Smoke Oxidative Stress; 2006: p. 5-46. http://dx.doi.org/10.1007/3-540-32232-9_2.
15. Horinouchi T, Higashi T, Mazaki Y, Miwa S. Carbonyl compounds in the gas phase of cigarette mainstream smoke and their pharmacological properties. Biol Pharm Bull. 2016;39(6):909–14. https://doi.org/10.1248/bpb.b16-00025.
16. Caliri AW, Tommasi S, Besaratinia A. Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. Mutat Res Mutat Res. 2021;787:108365. https://doi.org/10.1016/j.mrfmmm.2021.108365.
17. Mohammed B, Al-Thwani A. Evaluation the effect of nicotine injection on the lungs of mice. J Reports Pharm Sci. 2019;8(1):34–8. https://doi.org/10.4103/jrptps.jrptps_28_18.
18. Hiremagalur B, Nankova B, Nitahara J, Zeman R, Sabban EL. Nicotine increases expression of tyrosine hydroxylase gene. J Biol Chem. 1993;268(31):23704–11. https://pubmed.ncbi.nlm.nih.gov/7901211/.
19. Arabaci Ü, Akdur H, Yigit Z. Effects of smoking on pulmonary functions and arterial blood gases following coronary artery surgery in Turkish patients. Jpn Heart J. 2003;44(1):61–72. https://doi.org/10.1536/jhj.44.61.

20. Socol ML, Manning FA, Murata Y, Druzin ML. Maternal smoking causes fetal hypoxia: experimental evidence. Am J Obstet Gynecol. 1982;142(2):214–8. https://doi.org/10.1016/s0002-9378(16)32339-0.
21. Lykkesfeldt J. Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin Chim Acta. 2007;380(1–2):50–8. https://doi.org/10.1016/j.cca.2007.01.028.
22. Hamady JJ, Al-Okaily BN. Anti-inflammatory and antioxidant effects of zinc and vitamin D on nicotine-induced oxidative stress in adult male rats. Int J Health Sci (Qassim). 2022;6(June):73–91. https://doi.org/10.53730/ijhs.v6nS9.12171.
23. Guidet B, Shah SV. Enhanced in vivo H_2O_2 generation by rat kidney in glycerol-induced renal failure. Am J Physiol Physiol. 1989;257(3):F440–5. https://doi.org/10.1152/ajprenal.1989.257.3.f440.
24. Snedecor GW, Cochran WG. Statistical methods, 6th Edition. The Iowa State University Press; 1980. p. 238–48.
25. Raeeszadeh M, Beheshtipour J, Jamali R, Akbari A. The antioxidant properties of Alfalfa (Medicago sativa L.) in nicotine-induced oxidative stress in the rat liver. Oxid Med Cell Longev. 2022;2022: 2691577. https://doi.org/10.1155/2022/2691577.
26. Oyeyipo IP, Raji Y, Bolarinwa AF. Nicotine alters serum antioxidant profile in male albino rats. N Am J Med Sci. 2014;6(4):168. https://doi.org/10.4103/1947-2714.131240.
27. Hamza RZ, El-Shenawy NS. Anti-inflammatory and antioxidant role of resveratrol on nicotine-induced lung changes in male rats. Toxicol Reports. 2017;4:399–407. http://dx.doi.org/10.1016/j.toxrep.2017.07.003.
28. Khudair NT, Al-Okaily BN. Renal ameliorating effect of resveratrol in hydrogen peroxide induced male rats. Iraqi J Vet Sci. 2022;36(3):571–7. https://doi.org/10.33899/ijvs.2022.132149.2193.
29. Schulz J, Kramer S, Kanatli Y, Kuebart A, Bauer I, Picker O. Sodium thiosulfate improves intestinal and hepatic microcirculation without affecting mitochondrial function in experimental sepsis. Front Immunol. 2021;12:671935. https://doi.org/10.3389/fimmu.2021.671935.
30. Sanchez-Aranguren L, Grubliauskiene M, Shokr H, Balakrishnan P, Wang K, Ahmad S. Sodium Thiosulphate-Loaded Liposomes Control Hydrogen Sulphide Release and Retain Its Biological Properties in Hypoxia-like Environment. Antioxidants. 2022;11(11):2092. https://doi.org/10.3390/antiox11112092.
31. Ascar IF, Khaleel FM, Hameed AS, Alabboodi MK. Evaluation of Some Antioxidants and Oxidative Stress Tests in Iraqi Lung Cancer Patients. Baghdad Sci J. 2022;19(6 Suppl.):1466. https://doi.org/10.21123/bsj.2022.19.6.Suppl.1466.
32. De Koning MSLY, Assa S, Maagdenberg CG, Van Veldhuisen DJ, Pasch A, Van Goor H. Safety and Tolerability of Sodium Thiosulfate in Patients with an Acute Coronary Syndrome Undergoing Coronary Angiography: A Dose-Escalation Safety Pilot Study (SAFE-ACS). J Interv Cardiol. 2020;2020. https://doi.org/10.1155/2020/8832803.
33. McGeer P. Medical uses of Sodium thiosulfate. J Neurol Neuromedicine. 2016;1(3):28–30. https://doi.org/10.29245/2572.942X/2016/3.1094.