Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Potential neurobehavioral toxicity of sulfur in mice

F.S. Hassan and B.Kh. Al-Baggou

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Article information

Article history:
Received August 16, 2022
Accepted March 29, 2023
Available online June 08, 2023

Keywords:
Sulfur
Toxic effects
Neurobehavior
Mice

Correspondence:
B.Kh. Al-Baggou
banan1971@uomosul.edu.iq

Abstract

Sulfur is one of the crucial elements in animal nutrition and has an influential role in forming amino acids. It is a common toxic element and does not significantly threaten human and animal health. The current study aimed to examine the potential neurobehavioral toxicity of sulfur in mice by studying its effects on animal behavior and locomotor activity. The 24h median lethal dose- (LD_{50}) of sulfur in mice, as determined by the up-and-down method was 33.22 g/kg; orally, whereas its approximate lethal dose (ALD) was 36 g/kg, within 24 h. Signs of sulfur poisoning in mice were depression, gasping, Straub tail, Piloerection, tremor, dyspnea, muscle fasciculation, and convulsions followed by death. Sulfur at 4 and 8 g/kg doses, after 4 and 24 h, caused significant changes in the neurobehavioral performance in mice. It appeared as a significant decrease in motor activity within the open-field test (number of crossed squares and number of rearing within 3 minutes), with prolongation and increase in the time required to complete the negative geotaxis test with a significant reduction in the number of head pocking and a slight decrease in the swimming test scores. Repeated administration of sulfur at doses of 1, 2, and 4 g/kg; for 7 and 14 consecutive days also led to significant decrease in the motor activity inside the open-field with a prolongation of the time required to complete the negative geotaxis with a significant reduction in the number of head pockings and a slight reduction in the swimming endurance test. Using a pharmacological challenge with xylazine and ketamine, sulfur induced a delay and prolongation in the onset of time of sleep and shortened the sleep duration. This study concludes that sulfur, despite its low toxicity, is a potential neurobehavioral toxicant.

DOI: 10.33899/IJVS.2023.135244.2461,

Introduction

Sulfur is an essential non-metallic chemical element, found in nature in a raw form and has an influential role in the continuation of life on earth (1,2). It is an essential component of many nutritional compounds needed by humans, such as amino acids and proteins, and it is polymorphic. It can appear in the form of sulfates or sulfides and is obtained from fossil fuel burners or through emissions from the oceans due to the activity and decomposition of microorganisms in addition to volcanic eruptions (3,4). It also enters the production of metals, oil refining, the manufacture of fertilizers, explosives and pesticides, and sulfur also enters the production of some chemicals and is one of the main ingredients in the pharmaceutical industry (5). Sulfur poisoning occurs in farm animals, especially ruminants, because of its high concentration in food and healthy water and the extensive use of fertilizers in fields and farms (6). Sulfur factories and fields were exposed to burning in Nineveh Governorate, Iraq in 2003, 2016, 2019 which caused high environmental pollution with sulfur as a result of the emission of toxic gases, and these emissions were sources of threat to humans, animals, and plants. Because of the seriousness of these toxic emissions and the lack of scientific research on this environmental pollutant which is considered safe, we have highlighted the potential neurobehavioral toxicity of sulfur in mice.

Materials and methods

Ethical approval

The approval was obtained by the Scientific Council of the Faculty of Physiology, Biochemistry, and Pharmacology at the Faculty of Mosul's College of Veterinary Medicine and is a component of a master's thesis.

Animals

70 male and female; albino Swiss origin mice 25-38 g, were housed in plastic cages in an environmentally controlled animal house of the College of Veterinary Medicine, University of Mosul and maintained on a 10 h light/14 h dark cycle at temperature of 22±3 °C. Water and laboratory diet were available ad libitum.

Doses preparation

Distilled water and tween 80 were used to prepare the doses of sulfur. Tween 80 facilitated the dissolution and mixing of sulfur and the formation of a colloidal mixture (7) that was easily administered orally to mice. The administration volume was 10 ml/kg of body weight, orally.

The median lethal dose (LD_{50}) of sulfur in mice by the up-and-down method of Dixon

Preliminary experiments were conducted on mice to reach an appropriate dose used in our subsequent experiments on sulfur. Five mice (male and female) were used and- a dose of 32 g/kg b.wt of sulfur was chosen as the first dose (Because of the low toxicity of the elemental sulfur and the difficulty of dissolving and mixing it in 10 ml of distilled water and Tween 80, we divided the dose into 4 part of 8 g/kg each which was dosed every hour the LD50 of sulfur was determined by the up-and-down method. (8-10).

Approximate lethal dose (ALD) of sulfur in mice by the Diechman method

Four mice (male and female) were dosed with sulfur. The first dose was 16g/kg, orally, representing approximately 50% of the LD_{50} of sulfur. The mice were monitored within 2 h. after dosing, for the appearance of signs of poisoning, which were recorded accordingly. After 24 h, the result was read, which represented the survival or death of the mouse, and when the mouse survived, another mouse was dosed with the previous lethal dose multiplied by 1.5, and the same method was followed several times. The approximate lethal dose of sulfur is the first dose that kills the treated mouse (11).

Acute neurobehavioral effects induced by sulfur at the doses 4 and 8 g/kg b.wt orally in mice after 24 hours

Thirty mice (male and female) were used and divided in first group (control group) dosed with distilled water + Tween 80 in a volume of 10 ml/kg of b.wt, orally. Second group orally dosed with sulfur 4 g/kg of b.wt. Third group dosed orally with sulfur 8 g/kg of body weight. After 4 and 24 h of mice treatment, neurobehavioral and motor measurements were taken for each mouse in a quiet room.

Open-field activity test

The mice were individually subjected to an open-field test for 3 min. in a locally manufactured wooden box 35*35*25 cm. Its base was divided into 25 equal squares (12,13). The test measures general locomotor activity (squares crossed and rearing), within 3 minutes (Figure 1).

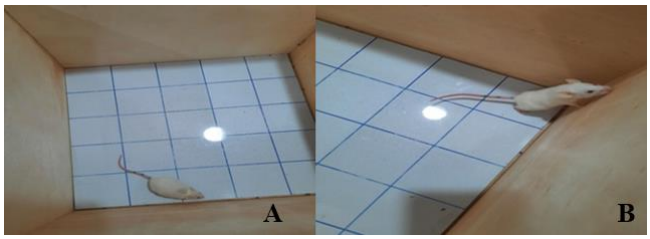

Figure 1: [A] Open-field activity test/ square crossed, [B] Open-field activity test/rearing.

Negative geotaxis test

This test measures neuromotor coordination and vestibular function in mice. The test is using a rough wooden surface inclined at 45° (14). Each mouse was placed in a head - down position and recorded the time needed to complete 180° turn, and we gave each mouse a maximum of 60 sec. to complete the task (Figure 2).

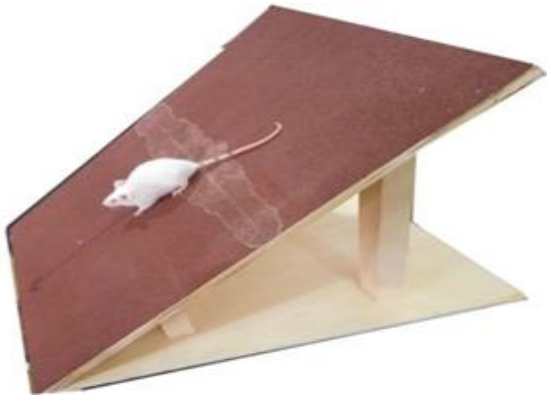

Figure 2: Negative geotaxis test.

Head poking test

This test measures the extent of curiosity and movement of the treated mouse. This test was carried out using a circular plastic surface with a diameter of 30 cm and a slight edge of 10 cm. It contains 8 circular holes with a diameter of 2 cm. The measurement begins by placing the mouse in the center of the circular surface and then counting the number of times the mouse inserts its head into the holes within 3 minutes (Figure 3) (15,16).

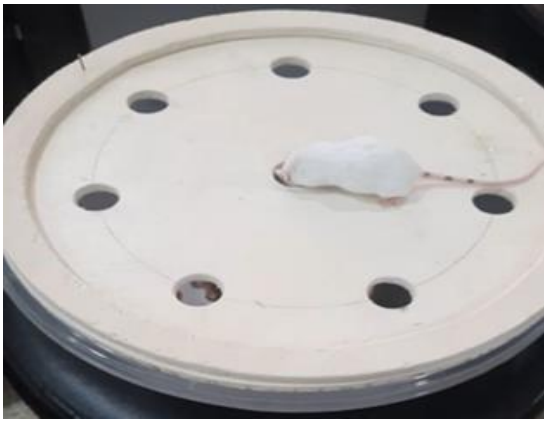

Figure 3: Head pocking test.

Swimming test

This test measures the extent to which the treated mouse responds to stress situations requiring coordination and balance between the CNS and the neuromuscular response. The test is done by placing each mouse in a glass basin with water at the height of 30 cm and the water temperature between 29-30°C,the mouse was placed in it for 5-10 seconds; and monitored while it was swimming, and then different swimming scores are recorded according to the degree of vulnerability of the treated mouse (Figure 4) (17,18). Swimming scores were as follows: Zero the nose is underwater. 1=The nose is at or above the water level. 2=Nose and vertex with or above level, while keeping the ears under the water. 3= Same as in 2 except that the water reaches the middle of the ear level. 4 = Same as in 3, except that the water reaches the base of the ear (Figure 4).

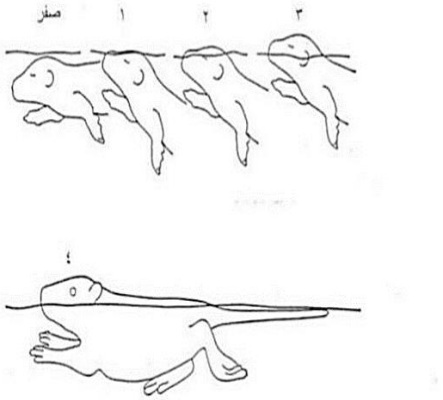

Figure 4: Swimming scores test.

Subacute neurobehavioral effects of sulfur at doses of 1, 2, and 4 g/kg b.wt in mice after daily administration for 7 and 14 consecutive days

28 mice (male and female) were divided into 4 groups as follows first group (control group) mice dosed with distilled water + Tween 80 orally. The second group was dosed orally with sulfur at 1g/kg b.wt. Third group mice were administered with sulfur 2 g/kg of b.wt orally. Four group mice were dosed with sulfur 4g/kg of b.wt orally. Mice were treated daily with sulfur for 6 consecutive days; and on the seventh day, the results of neurobehavioral and motor tests were recorded was open field activity test (the number of squares crossed and rearing). Negative geotaxis test. Head pocking test. Swimming endurance test. The test measures the mouse's ability to continue swimming and fatigue (19). In this test, the endurance capacity of the mouse was identified. The mice are placed in the swimming pool. Then time was recorded from the start of putting it in the tub until the appearance of signs of stress and cessation of swimming. After that, the mice were dosed with sulfur until day 13, and on day 14, the results of neurobehavioral and motor tests were recorded for the second time, as mentioned above.

The pharmacological challenge with xylazine and ketamine in mice treated with sulfur at the 4 and 8 g/kg b.wt orally

In order to reveal the latent and hidden effects of sulfur on the functions of the central nervous system and to show any imbalance that may occur in the central nervous system of mice, we used a mixture of xylazine and ketamine as a general anesthetic in mice (20,21). Thirty mice (male and female) were used. The mice were divided into 3 groups randomly. Each group contained 10 mice, which were treated as follows first group (control group) dosed orally with distilled water + Tween 80. Second group sulfur dosed 4 g/kg of b. wt orally. Third group dosed with sulfur 8 g/kg of body weight orally. After 30 minutes, xylazine 5 mg/kg b.wt + ketamine 50 mg/kg b.wt was injected intraperitoneally. After completing all the treatments, mice were monitored and the onset of sleep time (represented by loss of righting reflex) and sleep duration were recorded.

Statistical analysis

The parametric results were statistically analyzed by one and two-way analysis of variance, then subjected to the least significant difference test on the program Sigma plot (22). In contrast, the non-parametric results were analyzed using the Mann - Whitney U test at the level of significant difference for all tests, $P<0.05$.

Results

The median lethal dose (LD_{50}) of sulfur in mice by the Dixon method

LD_{50} of sulfur was 33.22 g/kg of b.wt orally. Signs of poisoning on sulfur-treated mice; were depression, gasping, piloerection, dyspnea, and tremor. Some animals showed muscle fasciculation, Straub tail, convulsion, and death in toxic doses (Table 1).

Table 1: LD_{50} of sulfur by oral administration in mice

Measurements	Result
The median lethal dose for sulfur	g/kg b.wt 33.22
Dosage range	48-16=32 g/kg b.wt
First dose	32 g/kg b.wt
Last dose	32 g/kg b.wt
Iincrease and decrease in the dose	4 g/kg b.wt
Table value amount	-0.305
Number of mice	5 (XOOXO)
Signs of poisoning	depression, gasping, tremor, dyspnea, muscle fasciculation, convulsions, and death

O: mouse survival within 24 hours. X: death of the mouse within 24 hours.

Approximate lethal dose (ALD) of sulfur in mice by diechman method

The lowest dose that led to signs of acute poisoning (depression, gasping, Straub tail, piloerection, tremor, dyspnea, muscle fasciculation, convulsions) and animal death within 24 hours was (36 g/kg) of b.wt orally.

Acute neurobehavioral effects induced by sulfur at the doses 4 and 8 g/kg b.wt orally in mice after4, 24 hours

Oral administration of sulfur at 4 and 8 g/kg b.wt doses caused neuromotor and neurobehavioral changes in mice after 4 hours of treatment. These doses reduced motor activity within the open field, which included a significant decrease in the number of squares crossed and rearing mice, within 3 minutes with both doses compared to the control group (Table 2).

Table 2: The open field activity in mice treated with sulfur at the doses of 4 and 8 g/kg of b.wt after 4 and 24 hours

Treatment	After 7 days	After 14 days
Open field activity/ squares crossed (3 min)		
Control	126.6±3.9	111.8±1.5
Sulfur 4g/kg	81±5.7	79.2±6.3*
Sulfur 8g/kg	101.8±6.6*AB	68.6±4.9*
Open field activity/ rearing (3 min)		
Control	26±2.8	22.4±1.9
Sulfur 4g/kg	20.4±3.6	19.6±1.7
Sulfur 8g/kg	24±2.5B	16±2.16*

* The value differs significantly from the control group at P<0.05. A The value differs significantly between the 4 g and 8 g groups at P<0.05. B The value varies significantly between 4 and 24 hours at P<0.05. Values are mean±SE.

When comparing the results of motor activity within the open field at the time 4 and 24 hours, there was a significant decrease in the number of squares crossed by mice treated with sulfur at a dose of 8 g/kg of b.wt during 4 and 24 hours (Table 2). Sulfur at a dose of 8 g/kg of b.wt caused a significant increase in the number of times rearing. The negative geotaxis test showed that oral administration of sulfur at the doses of 4 and 8 g/kg of b.wt caused a significant decrease in the time required to complete the test after 4 hours of treatment compared with the control group (Table 3). While there was a significant increase in the time required to complete the test by mice treated with sulfur at a dose of 8 g/kg b.wt after 24 hours of treatment and when comparing the results of this test between the two times 4 and 24 hours, there was a significant difference appeared in the time of test termination (Table 3).

In the Head pocking test (number of times the head is inserted into the holes), there was no significant change in both doses of 4 and 8 g/kg of b.wt after 4 hours of treatment compared to the control group, while the mice treated with sulfur at a dose of 8 g/kg of b.wt showed a significant decrease in the number of times after 24 hours of treatment compared to the control group. There was no significant change in the number times of inserting the head into the holes in the mice treated with both doses after 4 and 24 hours of sulfur treatment (Table 3). No significant change was observed in the results of the swimming test (scores). After 4 and 24 hours compared to the control group (Table 3).

Table 3: Neurobehavioral measurements (negative geotaxis, head poking, and swimming test) in mice treated with sulfur at 4 and 8 g/kg b.wt after 4 and 24 hours

Treatment	After 7 days	After 14 days
Negative geotaxis test (sec)		
Control	7±0.8	26±4.8
Sulfur 4g/kg	8±1.1	8±0.73*
Sulfur 8g/kg	13±4*A	12±2.5*
Head pocking (3 min)		
Control	37±3.3	26±2.8
Sulfur 4g/kg	27±3.7	23±4.6
Sulfur 8g/kg	24±1.7*	21±3.5
Swimming test (scores)		
Control	4±0	3.8±0.2
Sulfur 4g/kg	4±0	3.8±0.2
Sulfur 8g/kg	3.8±0.2	3.6±0.2

* The value differs significantly from the control group at P<0.05. A The value differs significantly between the 4 g and 8 g groups at P<0.05. Values are mean±SE.

Subacute neurobehavioral effect of sulfur at the doses of 1, 2and 4 g/kg b.wt in mice after 7 and 14 days

Seven days of repeated treatment with sulfur at doses of 1, 2, and 4 g/kg of b.wt caused a decrease in the number of squares crossed within 3 minutes, and the dose of 1 g/kg of b.wt showed a significant decrease in the number of squares compared to the control group, and after 14 days of treating the mice. The two doses of sulfur 1 and 2 g/kg b.wt caused a significant increase in the number of squares crossed by mice within 3 minutes compared to the control group (Table 4).

When comparing the results of open field activity between day 7 and day 14 of treatment, doses of 1, 2, and 4 g/kg of b.wt caused a significant decrease in the number of squares crossed within 3 minutes after 7 days compared to the number of squares crossed after 14 days of treatment. As for the results of the test of the number of rearing after 7 days of treatment, the three doses caused a significant decrease in the number of rearing compared to the control group, and the mice treated with the same dose also showed a significant decrease after 14 days in the number of rearing compared to the control group (Table 4).

Table 4: Open field activity in mice treated with sulfur at doses of 1, 2, and 4 g/kg b.wt after 7 and 14 days

Treatment	After 7 days	After 14 days
Open field activity/ square crossed (3 min)		
Control	80±3	51±1.3
Sulfur 1g/kg	42±2.8*	16±1.4*D
Sulfur 2g/kg	71±3.5A	30±0.5*D
Sulfur 4g/kg	65±3.5B	44.5±2.7BCD
Open field activity/ rearing (3 min)		
Control	23±0.7	20.4±0.9
Sulfur 1g/kg	13.4±1.25*	3.5±0.4*
Sulfur 2g/kg	12.9±1.9*	6.5±0.5*
Sulfur 4g/kg	14±1.9*	7±0.5*

* The value differs significantly compared with the control group, P<0.05. A The value differed significantly between the group treated with sulfur at a dose of 1 and 2 g/kg of b.wt at a P<0.05. B The value differed significantly between the group treated with sulfur at a dose of 1 and 4 g/kg b.wt at P<0.05. C The value differed significantly between the group treated with sulfur at a dose of 2 and 4 g/kg of b.wt at P<0.05. D The value differed significantly between the group treated with sulfur at 7 and 14 days at P<0.05. Values are mean±SE.

When conducting a negative geotaxis test, sulfur at doses of 1 and 2 g/kg of b.wt led to an increase in the time to complete the test compared to the control group after 7 days, while the dose of 4 g/kg of b.wt caused a slight decrease in the time to complete the test compared to the control group (Table 5). As for the results of this test after 14 days of treating mice, they showed different results, where a dose of 1 g/kg of b.wt caused a significant decrease in the time required to complete the test, and a slight decrease in the mice treated with a dose of 4 g/kg of b.wt compared to the control group while it worked The sulfur dose of 2g/kg b.wt significantly increased the time required to complete the test compared to the control group (Table 5).

When conducting the head pocking test, sulfur doses of 1, 2, and 4 g/kg of b.wt caused a significant decrease in the number of times inserting the head into the holes compared to the control group after 7 days of treating mice with sulfur. Additionally, the number decreased after 14 days in mice treated with doses of 1, 2, and 4 g/kg b.wt compared to the control group, as well as a significant decrease in the number of times when comparing the results between the times 7 and 14 days of treatment.

In a swimming endurance test in repeatedly treated mice with sulfur at doses of 1 and 4 g/kg of b.wt after 7 days of treatment, the mice showed a significant decrease in the time of swimming compared to the control group, and the mice treated with the three doses showed a significant decrease in the time of the test after 14 days of the treatment compared to the control group (Table 5). When comparing the results of the endurance test and swimming challenge in mice treated with sulfur between 7 and 14 days, there was a significant difference between the two doses 1 and 2 g/kg of b. wt (Table 5).

Table 5: Neurobehavioral measurements (negative geotaxis, head pocking and swimming endurance) in repeated treated mice with sulfur at 1,2 and 4 g/kg b.wt after 7 and 14 days

Treatment	After 7 days	After 14 days
Negative geotaxis test (sec)		
Control	5.9±0.5	10.1±0.7
Sulfur 1g/kg	10.7±1.3*	7±0.3*AD
Sulfur 2g/kg	10±1.4*	16.6±2.3*AD
Sulfur 4g/kg	4.3±0.4BC	9.8±0.5CD
Head pocking (3min)		
Control	36.5±2.0	21±81.2
Sulfur 1g/kg	20.2±2.4*	11.3±0.9*D
Sulfur 2g/kg	18.4±2.0*	7.8±O.5*D
Sulfur 4g/kg	19.5±0.9*	73±0.5*D
Swimming endurance test (sec)		
Control	83.8±2.5	95.3±1.8
Sulfur 1g/kg	30.3±2.9*	21.3±2*D
Sulfur 2g/kg	70.3±4.1A	60.1±1.6*AD
Sulfur 4g/kg	43.8±1.5*BC	38.2±1.4*BC

* The value differs significantly compared with the control group, P<0.05. A The value differed significantly between the group treated with sulfur at a dose of 1 and 2 g/kg of b.wt at P<0.05. B The value differs significantly between the group treated with sulfur at a dose of 1 and 4 g/kg of b.wt at P<0.05. C The value differed significantly between the group treated with sulfur at a dose of 2 and 4 g/kg of b.wt P<0.05. D The value differs significantly between the group treated with sulfur at a 7 and 14 days P<0.05. Values are mean±SE.

Pharmacological challenge with xylazine and ketamine in mice treated with sulfur at doses 4 and 8 g/kg of b.wt

Sulfur at doses of 4 and 8 g/kg of b.wt led to an increase in the onset of sleep time with a significant decrease in the sleep duration compared to the control group (Table 6).

Table 6: Sleep time induced by xylazine and ketamine in mice treated with sulfur at the two doses of 4 and 8 g/kg of b. wt.

Treatment	Sleep time onset (min)	Sleep duration (min)
Control group Second group	2±0	36±1.71
Third group Fourth group	*2.8±0.32	*27.4±1.43
Fifth group Sixth group	*2.8±0.2	A*22.5±0.73

* The value differs significantly compared with the control group at P<0.05. A The value differs significantly between the mice treated with 4 and 8 g/kg b.wt at P<0.05. Values are mean±SE.

Discussion

Sulfur is a slightly toxic element, widely used to control and liminate skin pests such as ticks and parasites that cause animals scabies. Also, it affects weed control, despite its many benefits in treating skin conditions and others. However, it may cause toxic adverse effects on human and animal health and may have residues in the environmental that harm living organisms (23,24). Sulfur poisoning occurs in farm animals, especially ruminants, because of its high concentration in food and well water and extensive use of fertilizers in fields and farms (23-26). Because of the high level of sulfur pollution and the lack of research studies, we decided to conduct this study on the potential neurobehavioral toxicity of sulfur in mice.

Our current study showed that in mice oral LD_{50} sulfur was 33.22 g/kg of b. wt. The signs of sulfur toxicity apparent in mice varied between gasping, Straub tail, piloerection, dyspnea, muscle fasciculation, tremor, and eventually death due to depression of the respiratory center in the brain, while previous studies showed that oral (LD_{50}) of sulfur was 5 g/kg of b.wt.

In mice and 2 g/kg b.wt in rabbits (27,28), and 3 g/kg b. wt. in rats (29,30). This difference may be attributed to the variations in animal species, solvents and compounds added to sulfur to obtain a colloidal compound to make it easy to be dosed in mice, and hence facilitate its absorption. Also, the Diechmann method obtained the approximate lethal dose (ALD) of sulfur, 36 g/kg. Of b.wt by oral administration to mice, a dose also considered high due to the low toxicity of sulfur This ALD result further supports the value of LD_{50} obtained in the present study (31-33).

A set of neurobehavioral tests have been selected that reflect some behavioral and motor functions, such as those we used to examine general motor activity within the open field (12-14), and neuromuscular balance (negative geotaxis test) based on self-rotation (14), the movement of curiosity (head pocking) (15,34) and the swimming endurance test and measurement of swimming scores (17). The current study of the effect of sulfur on the neuromotor behavior of mice at the doses of 4 and 8 g/kg of b.wt showed that it significantly reduced the number of squares crossed and rearing during 3 minutes inside the open field box due to the effect of sulfur on the nervous system directly, which made mice are in a state of inactivity, with a prolonged and significant increase in the time required to complete the negative geotaxis test, which showed a neuromuscular imbalance

And possibly vestibular dysfunction in mice. This test is considered a tool used to measure and study diseases of the nervous system and neurodevelopmental disorders, as it shows the effect on the brain and the lack of awareness of the mouse, this is in agreement with study on rats (12). The effect of sulfur on the exploratory function and the extent of the treated mouse's curiosity and movement, agrees with a previous study (35). As for the endurance test, the swimming challenge, and the measurement of swimming scores, which showed the extent of the treated mice's response to stressful situations that need regulation and balance between the central nervous system and the neuromuscular response, the sulfur-treated mice were not significantly affected by these doses. perhaps these doses were not effective enough This agrees with a study on mice (36,37). There is no evidence in scientific publications on the effect of sulfur in laboratory animals, on neuromotor behavior to address the toxic effect of sulfur because it is a safe element in nature (38,39). The experiment highlighted the integration of the CNS, and this test depends on the behavioral function to perform the functional challenges that the animal is required to face it in the neurobehavioral measurement. These experiments are not severed and do not cause signs of acute poisoning to the animals (40,41).

On the other hand the subacute neurobehavioral effect of sulfur after 7 and 14 days of repeated treatment of mice with doses of 1, 2, and 4 g/kg of b.wt orally showed a significant decrease in the number of squares crossed and the number of rearing within 3 minutes. This decrease is attributed to the toxic effect of sulfur on the central nervous system, which is in agreement with a previous study of the toxic effect of some toxic metals on the central nervous system (42,43). A dose of 4 g/kg of b.wt caused a slight decrease in the time required to complete the test at the end of the first week of treatment, and this may be due to the absorption of the sulfur in that dose less than the rest of the dose 1 g/kg of b.wt with an increase in time at a dose of 2 g/kg of b.wt accompanied by a decrease in the time required to complete the test in mice treated with a dose of 4 g/kg of body weight. As a result of the poisoning that occurred, as for the test of inserting the

head into the holes, there was a decrease in the number of head pocking with all doses given during the time 7 and 14 days of treatment as a result of the effect of sulfur on the nervous system by reducing movement and the mouse's curiosity in exploration (25). When conducting a swimming endurance test, there was a direct effect of sulfur on the brain of mice, which showed fatigue and lack of movement due to the toxic effect of sulfur on the brain of mice (43-45). Hence, the pharmacological challenge test was applied in our current study using a mixture of xylazine and ketamine, which produces general anesthesia in mice. Its results showed that treating mice with sulfur causes a prolongation in the onset of sleep and a decrease in sleeping time (the duration of sleep). In revealing the latent adaptive changes, it can be concluded initially that the pharmacological challenge dose not act on the same systems the toxicant affects. This finding is agreeing with another study on the sedative and hypnotic effects of xylazine and ketamine (20,46).

Conclusion

Even though sulfur is a safe and non-toxic element in high doses, we concluded from our study that sulfur could potentially cause many changes in the level of neuromotor behavior in mice and the level of pharmacological challenge.

Acknowledgment

Our study was supported by the College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Conflict of interest

The authors declare no conflict of interest regarding the present study.

References

1. Ejaz H, Bibi E, Ali W, Ahmad I, Lashari A, Faiz H, Nazar W. Sulfur and particulate matter affecting soil and underground plants. J Agric Appl Biol. 2022;3(1):40-49. DOI: 10.11594/jaab.03.01.05
2. FirZheng XY, Orellano P, Lin HL, Jiang M, Guan WJ. Short-term exposure to ozone, nitrogen dioxide, and sulfur dioxide and emergency department visits and hospital admissions due to asthma: A systematic review and meta-analysis. Environ Int. 2021;150:106435. DOI: 10.1016/j.envint.2021.106435
3. Parashar A, Kumar S, Dogra P, Parashar K, Singh A. Effect of nitrogen and sulfur applications on growth, yield and quality parameters of malt barley (*Hordeum vulgare L.*) varieties under semi-arid eastern plain of Rajasthan. Pharma Innov J. 2022;11(1):1765-1770. [available at]
4. Abiko Y, Katayama Y, Akiyama M, Kumagai Y. Lipophilic compounds in garlic decrease the toxicity of methylmercury by forming sulfur adducts. Food Chem Toxicol. 2021;150:112061. DOI: 10.1016/j.fct.2021.112061
5. Yu B, Yuan Z, Yang X, Wang B. Prodrugs of persulfides, sulfur dioxide, and carbon disulfide: important tools for studying sulfur signaling at various oxidation states. Antioxid Redox Signal. 2020;33(14):1046-1059. DOI: 10.1089/ars.2019.7880
6. Holland SL, Avery SV. Chromate toxicity and the role of sulfur. Metallomics. 2011;3(11):1119-1123. DOI: 10.1039/c1mt00059d
7. Okino S, Ikeo M, Ueno Y, Taneda D. Effects of Tween 80 on cellulase stability under agitated conditions. Bioresour Technol. 2013;142:535-539. DOI: 10.1016/j.biortech.2013.05.078
8. Dixon WJ. Efficient analysis of experimental observations. Ann Rev Pharmacol Toxicol. 1980;20:441-462. DOI: 10.1146/annurev.pa.20.040180.002301
9. AL-Najmawi T.K. and Al-Zubaidy M. H. 2022. Acute toxicity events of ivermectin in chicks model. Iragi Journal of Veterinary Sciences, vol 36, 4, pp 1119-1124).DOI: 10.33899/ijvs.2022.133188.2188.
10. Abdulrazzaq D. H. and Amin S.M. 2021. Acute toxicity of metronidazole and its interaction with chlorpyrifos in chicks. Iraqi J Vet Sci. 35,1. PP(13-18) DOI:10.33899/ijvs.2021.127035.1442.
11. Depass LR. Alternative approaches in median lethality (LD50) and acute toxicity testing. Toxicol lett. 1989;49(2-3):159-170. DOI: 10.1016/0378-4274(89)90030-1
12. Moser VC, McCormick JP, Creason JP, MacPhail RC. Comparison of chlordimeform and carbaryl using a functional observational battery. Fundam Appl Toxicol. 1988;11(2):189-206. DOI: 10.1016/0272-0590(88)90144-3
13. Molinengo L, Fundarò A, Orsetti M. The effect of chronic atropine administration on mouse motility and on ACh levels in the central nervous system. Pharmacol Biochem Behav. 1989;32(4):1075-1077. DOI: 10.1016/0091-3057(89)90085-3
14. Mohammad FK, St Omer VE. Behavioral and developmental effects in mice following in utero exposure to 2,4-D/2,4,5-t mixture. Neurobehav toxicol teratol. 1986;8(5):551-560. [available at]
15. McMillan DE. Risk assessment for neurobehavioral toxicity. Environ Health Perspect. 1987;76:155-161. DOI: 10.1289/ehp.8776155
16. Soni K, Parle M. Anxiolytic effects of *Trachyspermum ammi* seeds in mice. J Pharm Sci. 2017;3(1):71-74. DOI: 10.1166/jpsp.2017.1077
17. Schapiro S, Salas M, Vukovich K. Hormonal effects on ontogeny of swimming ability in the mice: Assessment of central nervous system development. Sci NY. 1970;168(3927):147-150. DOI: 10.1126/science.168.3927.147
18. Vorhees CV, Brunner RL, Butcher RE. Psychotropic drugs as behavioral temiceogens. Sci NY. 1979;205(4412):1220-1225. DOI: 10.1126/science.472738
19. Fushiki T, Matsumoto K, Inoue K, Kawada T, Sugimoto E. Swimming endurance capacity of mice is increased by chronic consumption of medium-chain triglycerides. J Nutr. 1995;125(3):531-539. [available at]
20. Short CE. Alph-2- agents in Animal sedation. Analgesia and Anasthesia. Santa Barbara: Veterinary Practice Publishing Co; 1992. 84 p.
21. Massey CA, Richerson GB. Isoflurane, ketamine-xylazine, and urethane markedly alter breathing even at subtherapeutic doses. J Neurophysiol. 2017;118(4):2389-2401. DOI: 10.1152/jn.00350.2017
22. Runyon RP. None parametric statics: A contemporary approach. New York: Addison Wesley Publishing Company; 1977. 42-870 p.
23. Sołek-Podwika K, Ciarkowska K, Kaleta D. Assessment of the risk of pollution by sulfur compounds and heavy metals in soils located in the proximity of a disused for 20 years sulfur mine (SE Poland). J Environ Manage. 2016;180:450-458. DOI: 10.1016/j.jenvman.2016.05.074
24. Chaudhuri SG. Pyritic sulfur—Its distribution, origin in coal seams and production of acid water in mines. In: Moitra AK, Kayal JR, Mukerji B, Bhattacharya J, Das AK, editors. In innovative exploration methods for minerals, oil, gas, and groundwater for sustainable development. USA: Elsevier; 2022. 273-293 p.
25. Beske PH, Wilhelm CM, Harvilchuck JA, Platoff Jr GE, Yeung DT. A rodent model of sulfur mustard hematologic toxicity for the efficacy evaluation of candidate medical countermeasures. Mil med. 2022;187(1-2):e106-e115. DOI: 10.1093/milmed/usaa510
26. Corvino A, Frecentese F, Magli E, Perissutti E, Santagada V, Scognamiglio A, Severino B. Trends in H2S-donors chemistry and their effects in cardiovascular diseases. Antioxidants. 2021;10(3):429. DOI: 10.3390/antiox10030429

27. Extoxnet A. Pesticide information profiles. Extension Toxicology Network. Copper Sulphate. 1996.
28. Cherdthong A, Khonkhaeng B, Seankamsorn A, Supapong C, Wanapat M, Gunn N, Polyorach S. Effects of feeding fresh cassava root with high-sulfur feed block on feed utilization, rumen fermentation, and blood metabolites in Thai native cattle. Trop Anim Health Prod. 2018;50(6):1365-1371. DOI: 10.1007/s11250-018-1569-8
29. Knight AP. The toxicology of sulfur and nitmicee in ruminants. Bov pract. 1985;121-123. [available at]
30. Hale AT, Brown RE, Luka Z, Hudson BH, Matta P, Williams CS, York JD. Modulation of sulfur assimilation metabolic toxicity overcomes anemia and hemochromatosis in mice. Adv Biol Regul. 2020;76:100694. DOI: 10.1016/j.jbior.2020.100694
31. Hall JO. Sulfur. In: Ramesh CG, editor. Veterinary toxicology. USA: Academic press; 2018. 483-487 p.
32. Bai X, Kavussanos MG. Hedging IMO2020 compliant fuel price exposure using futures contracts. Energy Econ. 2022;110:106029. DOI: 10.1016/j.eneco.2022.106029
33. Alrawe SA, Alzubaidy MH. Acute and Subacute toxicity effects of (ambda-cyhalothrin in chiks. Iraqi J Vet Sci. 2022;36(1):91-200. DOI:10.33899/ijvs.2021.129674.1678.
34. Al-Baggou' B. Neurobehavioral and biochemical changes induced by interaction between cadmium and some insecticides in mice [Ph.D. dissertation]. Mosul: University of Mosul; 2002.
35. Ruhela RK, Soni S, Sarma P, Prakash A, Medhi B. Negative geotaxis: An early age behavioral hallmark to VPA mice model of autism. Ann Neurosci. 2019;26(1):25-31. DOI: 10.5214/ans.0972.7531.260106
36. Renard CE, Dailly E, David DJ, Hascoet M, Bourin M. Monoamine metabolism changes following the mouse forced swimming test but not the tail suspension test. Fundam clin pharmacol. 2003;17(4):449-455. DOI: 10.1046/j.1472-8206.2003.00160.x
37. Ausma T, Bansal V, Kraaij M, Verloop AC, Gasperl A, Müller M, Kopriva S, De Kok LJ, van der Kooi CJ. Floral displays suffer from sulfur deprivation. Environ Exp Bot. 2021;192:104656. DOI: 10.1016/j.envexpbot.2021.104656
38. Weiss B. Neurobehavioral toxicity as a basis for risk assessment. Trends Pharmacol Sci. 1988;9(2):59-62. DOI: 10.1016/0165-6147(88)90118-6
39. Abou-Kassem DE, El-Abasy MM, Al-Harbi MS, Abol-Ela S, Salem HM, El-Tahan AM, El-Saadony MT, Abd El-Hack ME, Ashour EA. Influences of total sulfur amino acids and photoperiod on growth, carcass traits, blood parameters, meat quality and cecal microbial load of broilers. Saudi J Biol Sci. 2022;29(3):1683-1693. DOI: 10.1016/j.sjbs.2021.10.063
40. Alder S, Zbinden G. Neurobehavioral tests in single- and repeated-dose toxicity studies in small rodents. Arch Toxicol. 1983;54(1):1-23. DOI: 10.1007/BF00277811
41. Agrafiotis C, Thomey D, de Oliveira L, Ebert M, Gobereit B, Pernpeintner J, Sattler C. A particle receiver-driven thermochemical cycle employing elemental sulfur for solar thermochemical energy storage: Investigation of particles as concentrated sunlight harvesting media and sulphur trioxide splitting catalysts. Solar Energy. 2022;234:21-38. DOI: 10.1016/j.solener.2022.01.032
42. Yang Z, Liu ZW, Allaker RP, Reip P, Oxford J, Ahmad Z, Ren G. A review of nanoparticle functionality and toxicity on the central nervous system. J Res Soc Interface. 2010;7(4):S411-S422. DOI: 10.1098/rsif.2010.0158.focus
43. Anderson DR, Taylor SL, Fetterer DP, Holmes WW. Evaluation of protease inhibitors and an antioxidant for treatment of sulfur mustard-induced toxic lung injury. Toxicol. 2009;263(1):41-6. DOI: 10.1016/j.tox.2008.08.025
44. Olney JW, Ho OL, Rhee V. Cytotoxic effects of acidic and sulfur containing amino acids on the infant mouse central nervous system. Exp Brain Res. 1971;14(1):61-76. DOI: 10.1007/BF00234911
45. Abumounshar NM, Ibrahim S, Raj A. A detailed reaction mechanism for elemental sulfur combustion in the furnace of sulphuric acid plants. J Chem Eng. 2021;99(11):2441-2451. DOI: 10.1002/cjce.24185
46. Al-Zubaidy MH, Amin SM, Al-Sanjary DH. Neurotoxicity of xylazine in chicks. Iraqi J Vet Sci. 2023;37(1):289-296. DOI: 10.33899/ijvs.2020.127100.1458.

السمية السلوكية العصبية المحتملة للكبريت في الفئران

فراس صلاح حسن علي و بنان خالد البكوع

فرع الفسلجة والكيمياء الحياتية والأدوية، كلية الطب البيطري، جامعة الموصل، الموصل العراق

الخلاصة

يعد الكبريت أحد العناصر المهمة في تغذية الحيوان وهو ثالث اهم عنصر في الجسم وله دور مهم في تكوين الأحماض الأمينية، وهو عنصر منخفض السمية ولا يشكل خطر كبير على صحة الإنسان والحيوان. من هنا هدفت الدراسة الحالية معرفة الإمكانية السمية العصبية السلوكية للكبريت في الفئران من خلال دراسة التأثيرات التي يحدثها الكبريت على مستوى سلوك وحركة الفئران. تم تحديد الجرعة المميتة الوسطية للكبريت في الفئران بالاعتماد على طريقة دكسون، إذ بلغت ٣٣,٢٢ غم/كغم من وزن الجسم عن طريق الفم، بينما كانت الجرعة المميتة التقريبية للكبريت ٣٦ غم/كغم من وزن الجسم عن طريق الفم والتي أدت الى موت الفئران خلال ٢٤ ساعة، تمثلت علامات التسمم بالكبريت بالاكتئاب، صعوبة البلع، انتصاب الذيل، انتصاب الشعر، الرجفة، صعوبة التنفس، التحزم العضلي، الاختلاجات العصبية والموت. أدى إعطاء الكبريت بالجرعتين ٤ و ٨ غم/كغم من وزن الجسم الى إحداث تغيرات معنوية في الاختبارات السلوكية العصبية بعد ٤ و ٢٤ ساعة من التجريع عبر الفم في الفئران تمثلت بالانخفاض المعنوي في النشاط الحركي داخل الميدان المفتوح (عدد المربعات المقطوعة وعدد مرات الوقوف على القوائم الخلفية خلال ٣ دقائق)، مع إحداث إطالة في الوقت اللازم لإنهاء اختبار الانتحاء الأرضي السالب فضلا عن التخفيض المعنوي في عدد مرات إدخال الراس في الثقوب، من جهة أخرى أدى الإعطاء المتكرر للكبريت بالجرع ١ و ٢ و ٤ غم/كغم من وزن الجسم عن طريق الفم بعد ٧ و ١٤ يوم من المعاملة الى حدوث انخفاض معنوي أيضا في النشاط الحركي داخل الميدان المفتوح مع إحداث إطالة في الوقت اللازم لإنهاء اختبار الانتحاء الأرضي السالب مع التخفيض معنويا في عدد مرات إدخال الراس في الثقوب وخفض بسيط في اختبار تحدي السباحة، وعند إجراء تحدي دوائي للكبريت بالزيلازين والكيتامين، أحدث الكبريت إطالة في وقت بدء حدوث النوم وقصر مدة النوم. نستنتج من هذه الدراسة إمكانية وقدرة الكبريت على إحداث سمية سلوكية عصبية على عدة مستويات كالسلوك العصبي وعلى مستوى التحدي الدوائي في الفئران على الرغم من سميته الواطئة.