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Some Biochemical changes induced by Toxic Effects of Sulfur in Mice

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ABSTRACT

Sulfur is one of the most reactive chemical elements, The EPA (environmental protection agency) has labeled elemental sulfur as generally safe. This study sheds light on the ability of sulfur to cause toxic biochemical effects by measuring biochemical changes in many parameters. The activity of alanine aminotransferase (ALT) and aspartate aminotransferase(AST), Glutathione (GSH) and Malondialdehyde (MDA) and glucose in blood plasma, brain, and liver of mice. Mice were orally dosed with sulfur at doses of 4 and 8 kg b.wt. which significantly decreased blood sugar level, ALT, and AST activity at 8 g/kg .in blood plasma after 4 and 1 day. On the other hand, administration of sulfur at doses of 1 and 4 g/kg b.wt, after 7 and 14 days of repeated treatment with it led to a significant decrease in the level of GSH in blood plasma and liver of mice with a significant increase in the level of GSH in the brain, while the 3doses of sulfur caused a significant increase of MDA level in blood plasma, brain, and liver of treated mice. The results of our follow-up testing also showed the biochemical effects of sulfur on both ALT and AST enzymes; it showed a slight increase in the level of both enzymes in blood plasma and a significant decrease in the level of brain GSH after 24 hrs of treatment. In contrast, the level of brain GSH significantly increased after 14 days of sulfur dosing, with a significant increase in the activity of both enzymes(ALT, AST) which indicates the persistence of the toxic effect on the liver. We conclude from this study the possibility and ability of sulfur to cause toxic biochemical effects in mice.

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INTRODUCTION

Sulfur is one of the important non-metallic chemical elements, denoted by its symbol, the letter S, and it has a specific color that distinguishes it from other elements, which is yellow and may vary in color to orange or gray if it contains a group of impurities **(Madigan and Martino, 2006)**.

It is an essential component of many elements in human requirements, such as amino acids and proteins, and it is polymorphic. **(Ejaz *et al.*, 2022). S**ulfur can appear in the form of sulfates or sulfides and is obtained from fossil fuel burners or through emissions from the oceans, where such emissions are obtained as a result of the activity and decomposition of microorganisms in addition to the permanent and continuous eruption of volcanoes **(Parashar *et al.*, 2022).** It is one of the main ingredients in the pharmaceutical industry (Wang et al., 2022). It is also used in producing metals, oil refining, fertilizers, explosives, and pesticides **(Likus *et al.*, 2015).** 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 **(Holland and Avery, 2011)**.

Especially as a result of the emission of toxic gases from the combustion of sulfur factories and fields ،During the past years, the concentration of these pollutants has significantly increased, exceeding the permissible limits and exposing living organisms, the environment, air, water, and soil to high degrees of pollution as hidden effects that hide and appear later in the form of deadly diseases that threaten public health, for humans, animals, and plants. Due to the lack of research studies on sulfur toxicity and the lack of clarity on its acute and sub-acute biochemical toxic effects, we decided to conduct this study in mice.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Scientific Council of the Department of Physiology, Biochemistry, and Pharmacology at the College of Veterinary Medicine,

University of Mosul. This study is part of a master's thesis.

Animals

In this study, mice of swiss origin male and female aged 1-2 months, were used. Their weight ranged from 25-38 g. Mice were raised in the animal house of the College of Veterinary Medicine at the University of Mosul in laboratory conditions at a temperature of about 22±2 centigrade and a light cycle of 10 hours of light and 14 hours of darkness. The mice were provided with feed and water throughout the day.

Drugs

1. Pure sulfur powder, manufactured by Merch and Darmstadt, Germany.
2. Tween (80%) acts on forming a colloidal mixture and easily swallowed sulfur, made by Analyst, England.
3. Ether made by Thomas Baker Limited, England.

Preparation of sulfur

Sulfur doses were prepared by adding distilled water and tween 80 (10 ml/kg) to facilitate the mixing of sulfur (doses 4 and 8 kg) and the formation of a colloidal mixture that the mice could easily swallow. **(Okino *et al*., 2013; Pan *et al*., 2014).**

Experimental design

1.Effects of sulfur on blood glucose level, ALT, and AST activities in the mice

Thirty mice (males and females) were used, divided into three groups of 10 mice each and dosed as follows.
GroupI: (control group) given with distilled water and Tween 80 orally.
GroupII: orally administered mixed with tween 80 (1 ml) + DW (9 ml) and sulfur 4 g / kg of b.wt.
GroupIII: orally administered mixed with tween 80 (1 ml) + DW (9 ml) and sulfur 8 g / kg of b.wt.

After 4 and 24 hours of treatment, The mice were anesthetized and blood samples were collected from the plexus of an eye to measuring the glucose level in the blood plasma using a glucose meter (Kit) made by (USA), using a spectrophotometer at a wavelength of 546 nm.:The effects of oral administration of sulfur at 4 and 8 g/kg b.wt doses on the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the mice measured by using kits (USA).

2.Effect of sulfur on the levels of GSH and MDA in blood plasma, brain and liver of the mice

This experiment was designed to show the long-term effect of sulfur on the level of glutathione, which is the first line of defense against oxidative damage, in addition to measuring the level of malondialdehyde in the blood plasma, brain and liver of the mice. 28 mice (males and females) were divided into 4 equal groups; each group contained 7 mice and were treated as follows:

GroupI: (control group) mice were administered with + Tween 80 DW (1 ml) distilled water (9 ml) orally.
GroupII: mice orally administered mixed with tween 80 (1 ml) + DW (9 ml) and sulfur 1 g / kg of b.wt.
GroupIII:mice orally administered mixed with tween 80 (1 ml) + DW (9 ml) and sulfur 2 g / kg of b.wt.
GroupIV: mice orally administered mixed with tween 80 (1 ml) + DW (9 ml) and sulfur 4 g / kg of b.wt.

After repeated dosing of sulfur one time\day for 14 days, Mice were anesthetized with ether, and blood samples were collected from the plexus of the eye to frozen at -18 c until GSHandMDA levels were measured by the modified Manual method **(James *et al*., 1982).** Method of measuring malondialdehyde level: **(Ohkawa *et al*., 1979).** The measurement was carried out using the MDA kit, made in (USA)

3.Follow-up testing of sulfur-induced biochemical changes in mice(Feldman., 1999)

We used Follow up-testing or follow-up effects and biochemistry to reveal the long-lasting of these changes and the occurrence of recovery in mice after 14 days of treatment. 32 mice (males and females) were divided randomly into two groups with 16 mice for each group whose weights ranged between (28-34) treated as follows:
GroupI: (control group) mice were administered with + Tween 80 DW (1 ml) distilled water (9 ml) orally.
GroupII: mice orally administered mixed with tween 80 (1 ml) + DW (9 ml) and sulfur 16 g / kg of b.wt.

Then the mice were anesthetized, blood was drawn from the ocular plexus, and the plasma was sepamiceed and kept in a freeze at -18°C until measurements (ALT and AST) were performed on them using their measuring number. Then the brain tissue was sepamiceed by centrifugation to obtain a clear solution from the sample) to measure the level of glutathione in the brain tissue and after 14 days

Statistical analysis

Parametric results were analyzed statistically by one using the way analysis of variance and Two-way analysis of variance. The results were subjected to the Least significant difference test based on the statistical analysis program Sigma plot as if the level of significant difference for all tests at the level of probability ($P \leq 0.05$).

RESULTS

1. Sulfur effect on the Blood glucose level in the mice

Administration of the mice with a dose of 4 g/kg b.wt of Sulfur mixture significantly increased the level of blood glucose after 4 hrs, compared to the control groups while the 2 doses of 4 and 8 g kg. b.wt caused a significant increase in blood glucose level after one day compared to the control group (Table 1).

Table 1: Sulfur Effect on the blood level of glucose in the mice:

Treated group	The blood level of glucose (mg/100ml)	
	After 4 hour	After24 hour
Control	4.8±108,2	97.64±2.1
Sulfur 4g/kg	58.5 ± 5.2*	114.74 ± 3.8*
Sulfur 8g/kg	103.8 ± 4.4 A	103.8 ± 4.4*

*****:The value differs significantly from the control group at a level ($P \leq 0.05$).
A:The value differs significantly between the group treated with sulfur at a dose of 4 and 8 g / kg of b.wt at a level of ($P \leq 0.05$).

On the other hand, the two doses (4 and 8g/ kg b.wt.) of sulfur caused a significant decrease in the activity of ALT and AST in the blood plasma of the mice after 4 and 24 hrs of treatment. Compared to the control group. (Table 2)

Table 2: Sulfur effects on ALTandAST activity in mice:

Treatment groups	Enzyme activity (ALT) IU/Liter		Enzyme activity (AST) IU/L	
	After 4 hours	After 24 hours	After 4 hours	After 24 hours
Control	69.5± 4.8	77.8± 1.1	100.8± 3.4	147.1± 2.3
Sulfur 4g/kg	65.4± 3.2*	59.6± 3.8*	89± 3.1	132.4± 3.5
Sulfur 8g/kg	58.1± 2.0*	63.4± 4.0*	58± 3.3* A	B123.6± 4.9

*: The value differs significantly with the control group at a level of ($P \leq 0.05$) .
A: The value differs significantly between the group treated with sulfur at a dose of 4 and 8 g / kg of b.wt at a level of ($P \leq 0.05$).
B: The value varies significantly between 4 and 24 hours at a probability level of ($P \leq 0.05$).

2. Effect of sulfur on the level of Glutathione (GSH), Malondialdehyde (MDA) in Blood plasma, brain and liver of the mice

2.1. Sulfur at a dose of 2 g/kg b.wt for 14 days caused a significant decrease in blood plasma glutathione level compared to the control group, with a decrease rate of about 35.5 %. (Table 3) While the sulfur doses are 1 and 4 g / kg. b.wt Caused a no significant decrease in the level of glutathione in the blood plasma of the mice Compared to the control group with a decrease rate of about. 18.1 % and 23.5 % , respectively (Table 3).

Table 3: Sulfur effects on the plasma glutathione level in mice.

Treatment	Plasma glutathione level (µmol/ml)	Decreasing %
Control Distilled water + Tween 80	10.4 ± 0.4	
Sulfur 1g/kg	8.6 ± 0.1	18.1
Sulfur 2g/kg	6.8 ± 0.3*	35.5
Sulfur 4g/kg	8.0 ± 0.5	23.5

*: The value differs significantly compared with the control group at a level of ($P \leq 0.05$).

The Three Sulfur doses also caused a significant decrease in the level of GSH in the liver of the mice after 14 days of treatments with decrease rates of 34.2, 46.3, and 49.1 %, respectively (Table 4). Whereas sulfur in its three doses (1,2,4 g / kg, b.wt) significantly increased the level of glutathione in the brain of the mice after 14 days of treatment compared to the control groups with a rising rate of about 54.7, 76.3, and 35.7 %, respectively (Table 4).

Table 4: Sulfur effects on the brain and liver glutathione level in mice.

Treatment	Glutathione level in the brain (µmol/g of tissue)	% Rising	Liver glutathione level (µmol/g tissue)	% Decreasing
Control Distilled water+ Tween 80	7.1±1.0		19.3±2.5	
Sulfur 1g/kg	11.1±2.6*	54.7	12.8±3.3*	34.2
Sulfur 2g/kg	12.6±2.5*	76.3	10.4±2.6*	46.3
Sulfur 4g/kg	9.7±1.2* B	35.7	9.9±0.50*	49.1

*: The value differs significantly compared with the control groups at a level of ($P \leq 0.05$).
B: The value differed significantly between the groups treated with sulfur at a dose of 1 g and 4 g / kg of b.wt at a level of ($P < 0.05$).

2.2.The three sulfur doses (1, 2, 4g / kg.b.wt) also caused a significant increase in the MDA level in mice's blood plasma after 14 days of treatment, with a rising percentage of 51.1, 75.2, 52.1 % , respectively (Table 5).

Table 5: Sulfur effects on the Malondialdehyde level in the blood plasma of the mice.

Treatment	Malondialdehyde level in plasma (µmol/ml)	Rising %
Control Distilled water + Tween 80	2,0±0.3	
Sulfur 1g/kg	4.2±0.9*	51.1
Sulfur 2g/kg	A8.2±0,6*	75.2
Sulfur 4g/kg	4.3±0.9 B	52.1

*: The value differs significantly compared with the control group at a level of ($P \leq 0.05$).
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 level of ($P \leq 0.05$).
B: The value differs significantly between the group treated with sulfur at a dose of 1 and 4 g / kg of b.wt at a level of ($P \leq 0.05$).

Whereas the sulfur of 1 and 2g/kg. b.wt caused an increase in the MDA level in both the liver and brain tissues of mice. Only a Sulfur dose of 4 /kg b.wt caused a significant increase in the hepatic MDA Compared after 14hrs to the control treatment with a rising percentage of about 35.3 % (Table 6).

Table 6: Sulfur effect on the MDA level in brain and liver in mice.

Treatment	Malondialdehyde level in the brain (µmol/g of tissue)	Rising %	Malondialdehyde level in the liver (µmol/g of tissue)	Rising %
Control Distilled water +Tween 80	49.2±0.4		21.6±1.2	
Sulfur 1g/kg	50.4±0.6	2.43	29.3±0.9*	26.3
Sulfur 2g/kg	50.2±0.6	1.99	22.04±1.3 A	1.9
Sulfur 4g/kg	52,7±1.2	4.89	33.4±0.9* B	35.26

*: The value differs significantly compared with the control group at a level of ($P \leq 0.05$).
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 level of ($P \leq 0.05$).
B: The value differs significantly between the group treated with sulfur at a dose of 1 and 4 g/kg of b.wt at a level of ($P \leq 0.05$).

3.Follow-up testing of sulfur effect on some biochemical changes in mice

Follow up on the effect of sulfur after 14 h and 2 weeks (14 days) of giving it to the mice in a high dose (16g / kg .b.wt) for one time orally. The sulfur decreased the level of GSH in the brain significantly after one day and 14 days of treatment Compared to the Control group (Table 7).

Table 7: Effect of sulfur(16g / kg .b.wt)on brain GSH level after 24 h and14 day in mice.

Treatment	Glutathione level in mice brain (µmol/gm)	
Time	After 24 hour	After14 day
Control Distilled Water + Tween 80	22.3±2.3	19.6±1.1
Sulfur 16 g / kg of b.wt	16.8±1.7*	39.3±3.2* A

* : The value differs significantly compared with the control group at a level of ($P \leq 0.05$).
A: The value differed significantly between the group treated with sulfur at 24 h and14 days at a level of ($P \leq 0.05$).

The same dose of sulfur (16g/kg b.wt) caused a significant increase in the activity of ALT and AST enzymes in the blood plasma after both times (1 day and 14 days) of treatment (Table 8).

Table 8: Effect of sulfur (16g/kg b.wt) on the activity of ALY and AST Enzyme in plasma of mice after 24 hrs and 14 days.

Treatment	Enzyme activity (ALT) IU/L		Enzyme activity (AST) IU/L	
Time	After one day	After 14day	After one day	After 14days
Control Distilled Water + Tween 80	21.4± 1.5	17.2± 0.7	11± 0.5	16.5± 0.9
Sulfur at a dose of 16 g/kg Of body weight	23.5± 2.6	23.7± 1.8*	14,4± 0.6*	22.9± 1.4* A

*: The value differs significantly compared with the control group at a level of ($P \leq 0.05$).
A: The value differed significantly between the two times one day and 14 days in the mice treated with a dose of 16 g / kg of b.wt at a level of ($P \leq 0.05$).

DISCUSSION

In the present study, sulfur produces many changes in many biochemical parameters in the mice's plasma, brain, and liver. The administration of sulfur at the doses of 4 and 8g/kg.b.wt, after 4 hrs of treatment Caused a significant decrease in the blood glucose level as a result of its effect on the insulin secretion due to stress mice and this is consistent with a previous study **(Sugendran *et al*., 1992)**. While the same two doses caused a significant increase in blood glucose level after one day of treatment, perhaps because of a defect in the work of insulin and its protein receptors, where insulin signals and the function of pancreatic cells are impaired, causing a gradual deterioration in the level of blood glucose due to lack of beta cell stimulation and liver damage **(Withers *et al*., 1998)**.

Results also showed the effect of sulfur on liver enzymes after 4 and 24 hrs of treatment caused a slight decrease in the activity of ALT and AST in blood plasma at both times may be because sulfur did not affect the tissues and the liver of mice with these 2 low doses. This result agreed with the Previous study that there is damage in tissue at the level of the kidneys or a decrease in vitamin B6 metabolism in the treated mice **(Diehl *et al*., 1984)** or damage in hepatocytes which affects the protein synthesis inside the ribosome. Since the ALT and AST enzymes work to transport amino acids inside cells, and the linking of cysteine bonds to other protein bonds because it contains sulfur can be linked to another bond. That contains cysteine and alters the protein's activity, transforming ALT and AST enzymes into active enzymes **(Chen *et al*., 2020)**.

We studied the subacute effect of sulfur after 14 days of treated mice with doses of 1,2 and 4g / kg, b.wt, orally to detect the effect of sulfur on oxidative stress and understand the toxic effect of sulfur on tissues such as liver and brain. Glutathione is one of the important defense lines in the animal body against oxidative stress. It is the first line of defense against sulfur poisoning and works to reduce damage to liver and brain cells because it is a highly important antioxidant **(Stohs and Bagchi, 1995)**. Sulfur administration led to a significant decrease in GSH level in blood plasma after 14 days of treatment with doses of 1,2 and 4g/kg b.wt. with a reduction or decreased rate of 18.1 %, 35.5%, 23.5 %, respectively. This is consistent with previous studies showing that sulfur affects the internal organs of humans and animals. In the brain, an increase in the GSH level was noted, with a rate of raising between 35 % - 76 % due to its effect on the rats' cerebral cortex **(Gammon *et al*., 2001).** In the liver, macroscopic effects were also observed represented by yellowing of liver color, possibly due to the deposition of fat around it and the presence of icteric enlargement with yellowish spots in necrotic tissues **(Lamfon, 2007).** Oxidative stress is an increase in the number of free radicals within the organism's cells that occurs due to exposure to oxidizing compounds. This stress state indicates a defect in the vital cellular defenses of the antioxidant, such as glutathione inside the organism's cells. The state of oxidative stress by measuring the concentration of GSH and MDA levels in the plasma and tissues as the level of GSH decreases and is accompanied by a rise in the MDA level in cases of oxidative stress **(Patockova *et al*., 2003).**

Sulfur caused an increase in the level of MDA in the blood plasma, brain and liver of treated mice after 14 days; this agreed with the study mentioned that the effect of toxic metals showed a decrease in GSH level and an increase in the MDA level in rats, mice **(El-Demerdash, 2011; Pawar *et al*., 2016).** The percentage of rising in the blood plasma MDA level ranges between 51-75 %, while in liver tissue, a significant increase in the MDA Level was also observed with a lesser percentage of rising in the brain tissue; the reason may be attributed to the increase in the free radicals and damage in fats, proteins and amino acids in cells and cell membranes of liver tissue **(Milovanic *el al*., 2019).**

When we follow up on the toxic effects of sulfur after one day and 14 days of dosing, the mice with 16g / kg. b.wt, the effects of sulfur in the brain tissue appeared as a significant decrease in the level of GSH after 24 hrs and a significant increase in GSH level after 14 days of treatment; this effect may be attributed to the demise and expulsion of sulfur from the body of mice over time. The changes occurring in the brain tissue may be due to oxidative stress. This was confirmed by other studies in mice and fish **(Pawar *et al* ., 2016; Ezenwosuet *et al.*, 2021).**

Follow-up testing also showed a significant increase in the activity of both ALT and AST at one day and 14 days of treatment, which also may be due to liver damage and oxidative stress **(Afkami-Ardakaniand, 2013; Heydarnejed, 2013; Hosseini and Rezae, 2014)** which indicate a breakdown in hepatocytes and release of the liver enzymes and arrival in the plasma as a result of the stimulation of free radicals **(Yousef, 2003)** or perhaps the effect of sulfur on the level of the 2 enzymes, while causing simple hepatic necrosis **(Rao *et al*., 2022).**

CONCLUSION

It can be concluded that, the possibility of sulfur-producing toxic effects through its biochemical changes that appeared clearly in some enzymatic measurements in the blood plasma and on the level of GSH and MDA of the liver and brain. Also, when we

followed up on the effects of sulfur in mice after one day and 14 days, we concluded that the mice could recover from these effects, especially on the brain GSH.

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Conflict of interest

The authors declare that they are not involved in any potential conflicts of interest.

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