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# Low-Dose Hydrogen Sulfide Ameliorates Cisplatin-Induced Hepatotoxicity in Rats

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## ABSTRACT

**Objective:** Cisplatin (CIS) is non-specific to the cell cycle, has a cytotoxic effect, and is used in many cancers. The side effects of CIS, such as hepatotoxicity, seriously limit its clinical use. This experimental study aims to prevent hepatotoxicity, one of the clinical side effects that cisplatin may cause. At the same time, Hydrogen sulfide (H2S) will be applied prophylactically and therapeutically and its effect levels will be compared. This study aims to contribute to the literature by preventing dose restriction due to the hepatotoxicity side effect of cisplatin, which plays an important role in cancer treatment, by prophylactic application of H2S. Our study investigated the protective and therapeutic efficacy of sodium hydrosulfide (NaHS, a donor of H2S), which activates the antioxidant system on CIS-induced hepatotoxicity.

**Method:** Control (Vehicle), CIS (7.5 mg/kg CIS), H2S+CIS (10 µmol/kg NaHS+7.5 mg/kg CIS), CIS+H2S (7.5 mg/kg CIS+10 µmol/kg NaHS) groups were formed by using 35 rats in the study. At the end of the study, blood and liver tissue was taken, and histopathological and biochemical analyzes were performed.

**Results:** It was determined that sinusoidal dilatation and congestion increased significantly in the CIS group and decreased in the H2S+CIS and CIS+H2S groups. Likewise, glycogen loss occurred in the CIS group, and a significant improvement was observed in the H2S+CIS group. In addition, significant deterioration was detected in malondialdehyde, catalase, glutathione, aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase values in the CIS group compared to the control group. In contrast, significant improvements were observed in both the H2S+CIS and CIS+H2S groups compared to the CIS group.

**Conclusion:** Both protective and therapeutic beneficial effects of H2S in CIS-induced hepatotoxicity were demonstrated by histopathological and biochemical analyses.

**Keywords:** Cisplatin, Hepatotoxicity, Hydrogen Sulfide, Oxidative Stress, Rat

## Introduction

The liver has many essential roles, including the metabolism of nutrients, the synthesis of glucose and lipids, immunity, vitamin storage, and the detoxification of drugs and xenobiotics (Trefts et al., 2017; Koroglu et al., 2021). Drug-induced liver injury (DILI) is still known as the most common cause of acute liver failure in the West. The prevalence of DILI varies depending on geographic location, but the estimated annual incidence worldwide is between 1.3 and 19.1 per 100.000 exposed person (Garcia-Cortes et al., 2020). DILI can be severe and life-threatening in humans, resulting in significant morbidity/mortality and high healthcare costs (Amirana & Babby, 2015).

Cisplatin (CIS) is a widely used antineoplastic drug and is still used in the treatment of breast, cervical, esophageal, bladder, small cell lung, osteosarcoma, squamous cell carcinoma, and testicular cancer and has an important place in treatment protocols (Gao et

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al., 2021; Man et al., 2020). Although CIS is widely used, it has significant side effects. Its important side effects include nephrotoxicity, hepatotoxicity, cardiotoxicity, and its clinical use is limited due to these side effects (Neamatallah et al., 2018; Hassan et al., 2020; Darwish et al., 2017; Hwang et al., 2020). There are not many known pathways for CIS-induced hepatotoxicity, which began with an overabundance of reactive oxygen species (ROS) that caused oxidative stress, inflammation, DNA damage, and liver death (Abd Rashid et al., 2021). CIS causes oxidative/nitrosative stress-mediated damage and disruption of cellular function in many subcellular structures, especially lipids, proteins, and DNA in the cellular structure (Peres & da Cunha, 2013). In many experimental models, it has been suggested that CIS causes toxicity by disrupting the oxidative stress balance and increasing inflammation and apoptosis (Bentli et al., 2013; Lu & Cederbaum, 2006; Omar et al., 2016). As a result of the studies, it was determined that free radicals and (ROS) increased in the toxicity models induced by CIS, and it was shown that lipid peroxidation increased and glutathione levels decreased (Pratibha et al., 2006). However, the mechanisms of CIS-induced hepatotoxicity are not fully understood (Fathy et al., 2022).

Hepatotoxicity caused by CIS, which has widespread clinical use, necessitates discovering preventive and therapeutic agents (Al-Malki & Sayed, 2014). Hydrogen sulfide (H2S) is an endogenous gasotransmitter that can add a hydropersulfide moiety (-SSH) to the cysteine residue in target proteins and stimulate catalytic activity (Paul & Snyder, 2018). H2S, previously known as toxic, is a gas that acts as a regulator in many physiological processes as a result of recent studies (Feng et al., 2020; Yuan et al., 2017). It is known that H2S, in particular, plays a vital role in regulating hepatic physiology and pathology. Studies have shown that H2S has many protective and therapeutic (antioxidant, anti-inflammatory, antiapoptotic, cytoprotective) effects (Calvert et al., 2010). H2S, widely recognized and known as an antioxidant, has been shown to scavenge ROS directly (Yi et al., 2019). Although there are many sources of intracellular ROS, the most important source is the NADPH oxidase 4 (Nox4) based NADH/NADPH oxidase system (Crosas-Molist & Fabregat, 2015). Studies have also shown that it regulates signalling pathways in physiological functions such as kinase regulation and maintenance of mitochondrial ATP production (Cohen et al., 2013; Yan et al., 2013; Ye et al., 2020; Fu et al., 2012). Several studies have demonstrated the beneficial effects of H2S on endotoxemia, acetaminophen, microplastics, and nickel-induced liver damage (Fu et al., 2012; Li et al., 2021). NaHS (a donor of H2S) has been extensively applied in clinical trials to predict the biological effects of H2S (Li et al., 2015; Kimura, 2014).

Although many studies are showing the efficacy of H2S, its preventive and therapeutic efficacy in CIS-induced hepatotoxicity is unknown, yet (Azarbarz et al., 2020; Karimi et al., 2017; Ibrahim et al., 2022; Kwon et al., 2019; Tu et al., 2016).

This study aims to create a hepatotoxicity model in rats with cisplatin, a widely used antineoplastic drug, and to reveal the prophylactic and therapeutic effects of H2S, which has antioxidant, anti-inflammatory, antiapoptotic, cytoprotective, etc. effects.

## Methods

### Animals

Thirty-five Sprague Dawley rats (3–4 months, 200–250 g) were purchased from Inonu University Laboratory Animal Research Center. Rats were housed on a light and dark (12:12) cycle in a room with controlled temperature (21±3◦C) and humidity (60±3%). Rats were fed standard chow and normal water, and rats were given ad libitum access. During the experiment, animal care and all experimental procedures were performed following the National Institutes of Health Animal Research Guidelines and ARRIVE guideline 2.0 (Percie du Sert et al., 2020).

The protocol of the study was approved by the Animal Research Ethics Committee, Inonu University, Malatya, Faculty of Medicine (Protocol: 2015/A-84- 22.10.2015). The simple randomization technique formed the experimental groups. Also, all procedures performed during the experiment and evaluating the results obtained were studied blindly.

### Chemicals

The main component, CIS (Cisplatin DBL 100 mg/100 ml, Orna, Istanbul, Turkey) and NaHS (CAS number: 16721-80-5, Sigma-Aldrich, St Louis, MO) was purchased. Ketamine hydrochloride (Ketalar®) and Xylazine HCl (Alfamine®) were purchased from erse Medikal, Istanbul, Turkey.

### Experimental Design

For the experiment, 35 male Sprague Dawley rats were simply randomly divided into four groups and the experimental model is described in Figure 1. In our study, we used NaHS, the donor of H2S.

1. Control group (n=8): Rats were given 0.5 mL of NaCl 0.9 % solution via per-oral (p.o.) for 14 days (Hashmi et al., 2021).

2. CIS group (n=9): A single dose of 7.5 mg/kg CIS was administered to the rats via intraperitoneal (i.p.) (Wang et al., 2022).

3. H2S+CIS group (n=9): Rats were given a dose of 10 µmol/kg NaHS for 12 days via p.o. and a single dose of 7.5 mg/kg CIS intraperitoneally via i.p. on the 13th day (Otunctemur et al., 2014).

4. CIS+H2S group (n=9): After a single of 7.5 mg/kg CIS to rats via i.p. administration and the next 12 days, 10 µmol/kg NaHS was administered via i.p (Pan et al., 2009).

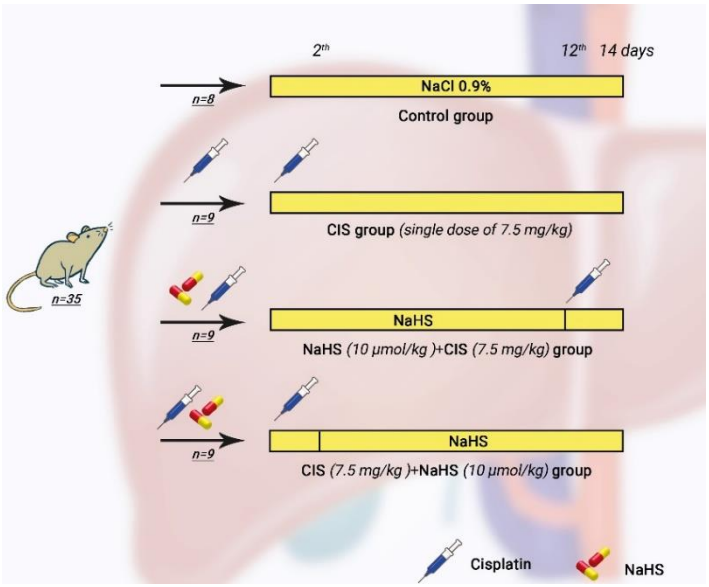


**Figure 1.** Schematic representation of the experimental design.

The body weights of the rats were weighed at the beginning and end of the study, as well as their liver weights at the end of the study. At the end of the experiment, all rats were sacrificed after ketamine and xylazine (75 mg/kg and 5 mg/kg, intraperitoneally) mixture administration. Immediately before the scarification procedure, blood samples were collected from the inferior vena cava for biochemical analysis. Hepatectomy was carried out after blood collection, and part of the liver sample was fixated with formalin for histopathological examination. The remaining tissues were stored at -70 ◦C for biochemical analysis. In addition, blood samples were taken into tubes without anticoagulant for the determination of liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH)]. Biochemical examinations [malonyldialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-Px)] and histopathological (light microscopy) were performed on liver tissue at the end of the experiment.

**Biochemical Analysis**

Liver The liver samples were homogenized (IKA ultra turrax T 25 basic) in cold phosphate buffer (pH 7.4). The homogenates were centrifuged (10000xg for 20 min at +4˚C), and the enzyme (MDA, SOD, CAT, GSH, and GSH-Px) levels determination was made in the supernatant part. The blood samples taken from rats were centrifuged at 2000xg for 10 min at +4˚C, and the obtained serum samples were used to measure the levels of AST, ALT, and LDH. The serum samples were frozen at -70˚C until assayed. The samples were taken to +4˚C one day before the biochemical analysis for correct analysis and thawing. AST, ALT, and LDH parameters were studied at Turgut Ozal Medical Center Laboratories (Abbott Architect c16000), Inonu University, Malatya, Turkey.

MDA, an indicator of lipid peroxidation, was studied according to the method of Uchiyama and Mihara (Mihara and Uchiyama, 1978). The rat liver sample was homogenized on ice for 1 minute at 15000 rpm to form 10% homogenate in 1.15% KCl solution. In the spectrophotometer, the absorbance of the supernatant was read at 535 nm. The result was shown as nmol/g tissue.

Tissue SOD activity was measured according to the method of Sun et al. (Sun et al., 1988). After adding 3 to 5 chloroform/ethanol mixture to the supernatants, all samples were centrifuged for 20 minutes at 5000 rpm at +4 degrees. Subsequently spectrophotometric evaluation of the samples at 560 nm was performed. Enzyme activity was given as U/g protein.

Tissue CAT activity was measured according to Luck's (De Bruijn, 1981) method. The rat liver sample was homogenized on ice for 1 min at 15000 rpm to form 10% homogenate. The absorbance at 240 nm was read immediately after the supernatant was added to the sample tubes. Enzyme activity was given as K/mg protein.

GSH was determined according to the method of Ellman (Ellman, 1959). The rat kidney sample was homogenized on ice to form 10% homogenate at 15000 rpm for 1-2 min. Then, the homogenate was centrifuged at 3000 rpm at +4 degrees for 15 min. Then, after adding TCA solution to the supernatant, it was mixed homogeneously and

centrifugation was repeated. After the protocol was completed, the samples were read at 410 nm in the spectrophotometer. GSH levels were presented as nmol/g tissue.

The GSH-Px activity was also determined according to the method of Paglia and Valentine (Paglia and Valentine, 1967) and measured by monitoring its oxidation at 340 nm. GSH-Px levels were presented as U/g protein).

**Histopathological Analysis**

At the end of the experiment, the liver tissue was fixed in 10% formaldehyde. After the tissue follow-up procedures, 4-5 µm thick sections were taken from the paraffin blocks prepared. Hematoxylin-eosin (H-E) staining methods were applied to the areas for general histological evaluations, and periodic acid schiff (PAS) staining methods were applied to determine glycogen loss. Sections using the H-E staining method, sinusoidal dilatation, sinusoidal congestion, and necrosis were examined. Damage according to its severity, ten randomly selected areas were evaluated by scoring 0 (no injury), 1 (light injury), 2 (moderate injury), and 3 (serious injury). In the PAS staining method, ten randomly selected areas were examined, and according to the prevalence of glycogen loss, 0; normal, 1; less than 25% change, 2; the difference between 25-50%, 3; It was evaluated by scoring with more than 50% change (Bilgic et al., 2018). Leica DFC-280 microscope was used for histopathological analysis and Leica Q Win Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, UK) was used for imaging analysis.

**Data Analysis**

Statistical power analysis was performed to determine the required power and sample sizes. Considering type I error (alpha) 0.05, power (1-beta) 0.8 and effect size 0.92 for AST levels, it was determined that the minimum sample size required to detect a significant difference should be at least 8 in each group (Arslan et al., 2018). The normality of the distribution was verified using the Kolmogorov-Smirnov test. Mann-Whitney U test was used for intergroup comparisons in histopathological analyzes. Post-hoc Tukey's test was applied after ANOVA to compare groups in biochemical analyses. Statistical evaluation of histopathological and biochemical analyzes was performed using SPSS (IBM SPSS Corp., Armonk, NY, USA) for Windows version 25. $p < .05$ was accepted as the significance level.

## Results

Mortality, Weight, and Liver-Body Weight Gain Ratio

Two rats in the CIS group and one rat in the CIS+ H2S group died due to toxicity caused by CIS during the drug administration period. At the end of the experiment, there was a statistically significant decrease in rat and liver weights in the CIS, H2S+CIS, and CIS+H2S groups compared to the control group ($p < .05$). Although there was no statistically significant difference in rat and liver weights in the H2S+CIS and CIS+H2S groups compared to the CIS group, an improvement tendency was detected. In contrast, no statistically significant difference was observed between the groups in terms of liver/rat weight ($p > .05$). Mortality, weight, and rate of increase in organ-body weight are presented in the Table 1.

**Table 1.** Descriptive statistics for liver, rat weight, and liver/rat weight ratios

| Groups | Rat weight-Before (g) | Rat weight-After (g) | Liver weight (g) | Liver/rat weight ratio |
|---|---|---|---|---|
| **Control (*n*=8)** | 308 (202-342) | 316 (276-343) | 10.57 (9.72-12.46) | .037 (.03-.043) |
| **CIS (*n*=7)** | 255 (219-330) | 207[a] (172-289) | 7.49[a] (7.08-11) | .038 (.025-.051) |
| **$H_2S$+CIS (*n*=9)** | 300 (268-355) | 250[a] (190-300) | 9.485[a] (6.17-11.3) | .039 (.025-.05) |
| **CIS+$H_2S$ (*n*=8)** | 325 (280-360) | 253[a] (210-332) | 8.605[a] (7.1-11.57) | .034 (.025-.055) |
| ***p*** | .05479 | .00332 | .01409 | .913 |

g: Gram.

**Biochemical Finding**

**Liver function tests**

There was a significant increase in AST, ALT, and LDH levels in the CIS group when compared to the control group, and a significant decrease in the H2S+CIS group and CIS+H2S group compared to the CIS group ($p < .05$). Serum biochemistry results are presented in the Table 2.

**Tissue biochemical findings**

There was a significant increase in MDA level in the CIS group compared to the control group, and a significant decrease in the H2S+CIS group and the CIS+H2S group compared to the CIS group ($p < .05$). There was a significant decrease in CAT and GSH levels in the CIS group compared to the control group, and a significant increase in the H2S+CIS group and CIS+H2S group compared to the CIS group ($p < .05$). No significant difference was found between the groups in terms of SOD and GSH-Px levels ($p > .05$). Liver tissue biochemistry results are presented in the Table 3.

**Table 2.** Serum biochemical parameters.

| Parameters | Groups | | | |
|---|---|---|---|---|
| | **Control (*n*=8)** | **CIS (*n*=7)** | **$H_2S$+CIS (*n*=9)** | **CIS+$H_2S$ (*n*=8)** |
| **AST (U/L)** | 163.38 ± 32.40 | 342.75 ± 96.65[a] | 155.13 ± 68.98[b] | 158.88 ± 52.83[b] |
| **ALT (U/L)** | 78.00 ± 12.39 | 215.63 ± 160.17[a] | 61.38 ± 24.10[b] | 72.13 ± 20.38[b] |
| **LDH (U/L)** | 730.38 ± 224.00 | 1560.63 ± 613.58[a] | 737.13 ± 403.10[b] | 657.88 ± 251.43[b] |

[a]$p < .05$: Significant compared to the control group.

[b]$p < .05$: Significant compared to CIS group.

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase.

**Table 3.** Biochemical parameters of liver tissue**.**

| Groups | Parameters | | | | |
|---|---|---|---|---|---|
| | **MDA (nmol/g tissue)** | **SOD (U/g protein)** | **CAT (K/mg protein)** | **GSH (nmol/g tissue)** | **GSH-Px (U/g protein)** |
| **Control (*n*=8)** | 141.75 ± 21.33 | 325.50 ± 206.20 | 2.77 ± 1.13 | 1226.75 ± 214.33 | 212.25 ± 111.58 |
| **CIS (*n*=7)** | 233.13 ± 40.90[a] | 344.13 ± 62.77 | 0.89 ± 0.54[a] | 788.88 ± 271.01[a] | 241.38 ± 118.01 |
| **$H_2S$+CIS (*n*=9)** | 155.75 ± 30.51[b] | 313.63 ± 101.36 | 2.66 ± 1.07[b] | 1263.00 ± 287.6[b] | 196.00 ± 97.98 |
| **CIS+$H_2S$ (*n*=8)** | 145.50 ± 35.92[b] | 341.63 ± 137.60 | 2.60 ± 1.24[b] | 1267.88 ± 379.6[b] | 219.38 ± 136.69 |

[a]$p < .05$: Significant compared to the control group.

[b]$p < .05$: Significant compared to CIS group.

MDA: Malonyldialdehyde; SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; GSH-Px: Glutathione peroxidase.

### Histopathological Findings

The histological structure of the liver had a normal appearance in the control group. In sections stained with H-E, hepatocyte cords anastomoses around central veins and sinusoids between these cords were clearly observed (Figure 2-A). PAS (+) staining was observed in the cytoplasm of hepatocytes due to glycogen accumulation (Figure 3-A). It was observed that sinusoidal dilatation and congestion increased significantly in the CIS group (Figure 2-B and C). In addition, necrotic areas were observed around the central vein in some sections in the CIS group (Figure 2-B). In the sections where the PAS staining method was applied, it was observed that PAS (+) areas decreased due to glycogen loss, especially around the portal area in the CIS group (Figure 3-B). Sinusoidal dilatation and congestion were observed to be statistically significantly decreased in the H2S+CIS and CIS+H2S groups compared to the CIS group ($p < .05$) (Figure 2-D and E). On the other hand, it was observed that glycogen loss decreased statistically significantly in the H2S+CIS group compared to the CIS group ($p < .05$) (Figure 3-C), but it was observed that it continued in the CIS+H2S group similarly to the CIS group ($p > .05$) (Figure 3-D). There was no statistical difference between the CIS+H2S group and the H2S+CIS group in terms of sinusoidal dilatation, congestion and necrosis ($p > .05$). However, it was observed that glycogen loss was higher in the CIS+H2S group than in the H2S+CIS group ($p < .05$). Also, histopathological analysis scores on liver tissue are given in Table 4.

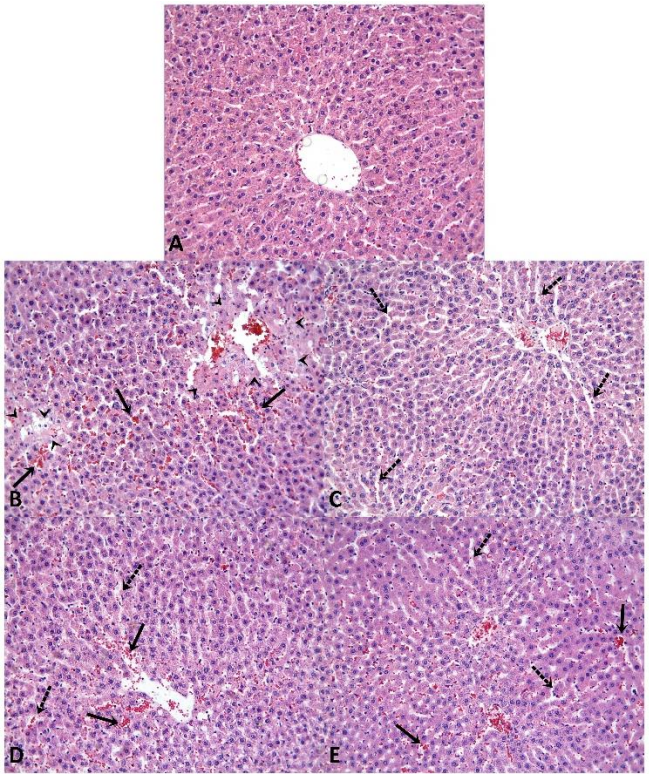


**Figure 2.** Liver tissue in the control group (A) has a normal histological appearance. Necrotic areas (arrowheads), sinusoidal congestion (arrows) and dilatation (dashed arrows) are observed around the central vein in the CIS group (B and C). It is observed that histological changes in the H2S+CIS (D) and CIS+H2S (E) groups are milder than the CIS group.

**Table 4.** Descriptive statistics for histopathological scores.

| Parameters | Groups | | | |
|---|---|---|---|---|
| | **Control (*n*=8)** | **CIS (*n*=7)** | **$H_2S$+CIS (*n*=9)** | **CIS+$H_2S$ (*n*=8)** |
| **Sinusoidal Dilation** | 0.0 (0.0-1.0) | 1.0 (0.0-3.0)[a] | 1.0 (0.0-3.0)[b] | 0.0 (0.0-2.0)[b] |
| **Sinusoidal Congestion** | 0.0 (0.0-2.0) | 1.0 (0.0-3.0)[a] | 0.0 (0.0-2.0)[b] | 0.0 (0.0-2.0)[b] |
| **Necrosis** | 0.0 (0.0-0.0) | 0.0 (0.0-3.0)[a] | 0.0 (0.0-0.0)[b] | 0.0 (0.0-0.0)[b] |
| **Glycogen Loss** | 1.0 (0.0-1.0) | 1.0 (0.0-3.0)[a] | 0.0 (0.0-3.0)[b] | 1.0 (0.0-3.0)[c] |

[a] $p < .05$: Significant compared to the control group.

[b] $p < .05$: Significant compared to CIS group.

[c] $p < .05$: Significant compared to the $H_2S$+CIS

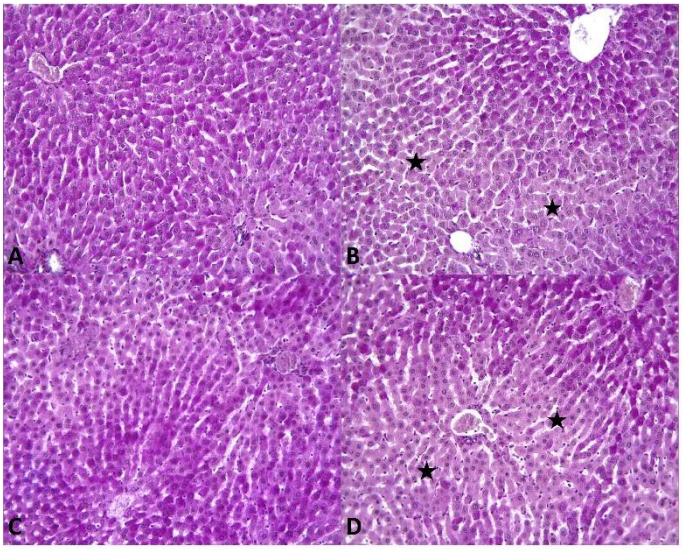


**Figure 3.** PAS (+) staining in hepatocytes in control group (A) indicates the presence of glycogen. In the CIS group (B), a decrease in PAS (+) staining is observed due to glycogen loss. In the H2S+CIS group (C), PAS (+) staining increased, but glycogen loss was similar to the CIS group in the CIS+H2S group (D).

## Discussion

This study aims to reveal both the therapeutic and prophylactic effects of low-dose H2S in the CIS-induced hepatotoxicity and evaluate it biochemically and histopathologically.

CIS, an antineoplastic drug, is widely used in the treatment of breast, cervical, esophageal, bladder, small cell lung, osteosarcoma, squamous cell carcinoma and testicular cancer (Gao et al., 2021; Man et al., 2020). However, the clinical application of CIS is limited due to its serious side effects such as hepatotoxicity (El-Gizawy et al., 2020). Furthermore, because of the harmful effects of ROS by exhausted GSH, antioxidant enzymes like glutathione GPx, SOD, CAT, GSH, and an increase in hepatic MDA, oxidative stress is a major factor in CIS-induced hepatotoxicity (Abd Rashid et al., 2021). In this current study, we focused on the improvement properties of H2S administration in hepatotoxicity caused by CIS.

A single dose of 7.5 mg/kg CIS not only caused an increase in liver function enzymes but also caused histopathological changes in this study. The increase in the level of liver enzymes in the serum indicates that the enzymes pass from the cytosol to the systemic circulation due to the deterioration of the integrity of the liver cell. Similar to the literature, our study observed an increase in liver enzymes (AST, ALT, and LDH) after CIS application, indicating liver damage (Neamatallah et al., 2018; Ijaz et al., 2020; Taghizadeh et al., 2021). In addition, as a result of histopathological evaluations in liver tissue, it was determined that CIS injection caused sinusoidal dilatation, congestion, necrosis, and glycogen loss. All these findings were in agreement with previous studies (El-Gizawy et al., 2020; Wang et al., 2018; Sherif, 2021). The integrity of the sinusoidal structure is extremely important in that it plays an important role in the continuous physiological exchange of metabolites and fluids in the liver. Disruption of this structure reflects the impairment of liver function (Ahmed, 2013). Also, similar to the literature, a decrease in body weight and liver tissue weight was detected in the CIS group compared to the control group (Ko et al., 2014; Maheshwari et al., 2015).

According to previous studies, it has been revealed that the use of some natural compounds and products improves both biochemical and histopathological damage caused by liver toxicity (El-Gizawy et al., 2020; Wang et al., 2018; Sherif, 2021). Our present findings showed that H2S provides significant improvements in histopathological deterioration in the hepatotoxicity model induced by CIS. In addition, H2S has been shown to have hepatoprotective and therapeutic effects. Also, the use of NaHS, an H2S donor, caused a significant decrease in liver enzymes and statistically significantly improved the histopathological damage caused by CIS in the liver. By replenishing the amount of antioxidant enzymes and functioning as an anti-inflammatory agent, a variety of natural products, plant extracts, and oil rich in flavonoids, terpenoids, polyphenols, and phenolic acids were able to reduce oxidative stress. Similarly, following CIS administration, honey and royal jelly therapy was shown to reduce serum transaminases and scavenge free radicals in the liver. These natural items' medicinal qualities show promise as a supplemental treatment to combat the hepatotoxicity caused by CIS (Abd Rashid et al., 2021).

The liver plays a central role in preventing damage caused by ROS and eliminating ROS products due to its detoxification properties and antioxidant capacity (Koroglu et al., 2021). Although there are many mechanisms underlying the damage caused by CIS in the liver, oxidative stress and decreased antioxidant capacity are the most important mechanisms (Al-Malki & Sayed, 2014). ROS, which is produced much more than the antioxidant capacity, shows many effects in the cell. These effects include an increase in cell death, a decrease in proliferation as well as an increase in lipid peroxidation (Gunata & Parlakpinar, 2021). All these changes affect the signaling pathways in the cell and cause hepatocyte damage (El-

Gizawy et al., 2020; Auten & Davis, 2009). It is known that CIS causes toxicity by increasing intracellular reactive oxygen and nitrogen species in the liver (Chirino et al., 2008). Overproduced free radicals damage not only intracellular structures but also highly unsaturated fatty acids in the cell membrane and may increase lipid peroxidation levels. Data from this study are consistent with previous studies supporting oxidative stress and lipid peroxidation (Fathy et al., 2022; Gunata & Parlakpinar, 2021; Chirino et al., 2008; Farooqui et al., 2016; Eisa et al., 2021).

In accordance with the results of the study in the literature, it was shown that CIS caused a significant decrease in the level of antioxidant enzymes such as CAT and non-enzymatic antioxidant GSH in the liver tissue. It caused a significant MDA value increase, indicating lipid peroxidation (Fathy et al., 2022; Taghizadeh et al., 2021; Sioud et al., 2020; Aboraya et al., 2022; Bilgic et al., 2018). MDA, which is accepted as a marker for oxidative stress, is a lipid peroxidation product and an increase in its level is an indicator of liver damage (Ko et al., 2014). In addition, the most important components of antioxidant defense in the intracellular structure include SOD, which catalyzes the conversion of superoxide anion radical to hydrogen peroxide and oxygen, and CAT, which hydrolyzes hydrogen peroxide to water and oxygen (Sherif, 2021). In addition, GSH has an antioxidant effect by interacting directly with ROS through its sulfhydryl group (Ko et al., 2014). In addition, reduced glutathione, known as an antioxidant, is an important endogenous antioxidant that acts by scavenging free radicals directly or through some antioxidant enzymes (Birk et al., 2013). Accordingly, the level of GSH in the CIS-administered group decreased as a result of its consumption in the scavenging of ROS and nitrogen species produced by CIS in this study. In addition, CIS causes a GSH-platinum complex to form upon entry into the cells (Chu, 1994). This causes a decrease in the level of GSH. The reason for the increase in the level of GSH in the liver may be the increase in the activity or expression of glutamylcysteine ligase, the rate-limiting enzyme in GSH synthesis (Dickinson et al., 2003). Activation of all these enzymes following CIS application leads to the formation of high levels of free radicals. Usually, ROS elimination occurs depending on the liver's antioxidant capacity (Koroglu et al., 2021). However, excess oxidant products from CIS administration exceed the antioxidant capacity of the liver. The reason for the increase in the level of GSH in the liver may be the increase in the activity or expression of glutamylcysteine ligase, the rate-limiting enzyme in GSH synthesis (Dickinson et al., 2003). However, there was no significant difference in SOD and GSH-Px values between all groups.

It has been shown that H2S improves the levels of antioxidant enzymes and non-enzyme CAT and GSH in the liver and reduces lipid peroxidation by causing a decrease in MDA value in CIS-induced hepatotoxicity. Previous studies have shown that H2S effectively scavenges ROS such as H2O2, O2•, and •OH and inhibits NADPH oxidase, which is the main source of ROS overproduction (Wang et al., 2019; Bitar et al., 2018). This may also explain the effect of H2S in current research, where it has been shown that H2S provides a significant decrease in hepatic MDA levels, the end product of lipid peroxidation, an increase in CAT, GSH levels, improvement in liver function enzymes (AST, ALT, LDH) and histopathological improvement.

Light microscopy was used to detect morphological changes in the liver. Our histopathological results showed that CIS treatment caused sinusoidal dilatation, congestion and necrosis. In addition, PAS staining was also performed, and it was observed that PAS (+) areas decreased due to glycogen loss in the CIS group. These effects are similar to the results of previous studies in the literature (Fathy et al., 2022; Eisa et al., 2021; Fatima et al., 2021; Coskun et al., 2021; Cagin et al., 2015). All of these findings explain the hepatotoxicity pathophysiology of CIS. On the contrary, it was determined that H2S treatment caused a statistically significant improvement in histopathological damage and PAS staining in both the prophylactic and treatment groups. This finding supports that HS treatment may have potent antioxidant effects in CIS-induced hepatotoxicity in rats. In conclusion, histopathological evidence supports our biochemical findings.

The normalization of these values in the prophylactic and therapeutic groups indicates that H2S has an antioxidant effect and has positive effects on liver damage caused by CIS. However, the protective and therapeutic efficacy of H2S was first reported in this study by histopathological and biochemical analyzes in the CIS-induced hepatotoxicity model.

## Conclusion

It has been shown that HS provides significant improvement in liver function enzymes (AST, ALT, and LDH), tissue antioxidant capacity (MDA, CAT, and GSH), and improvement in histopathological damage (sinusoidal dilatation, congestion, necrosis, and glycogen loss). The administration of H2S showed both prophylactic and therapeutic effects in the experimental model of hepatotoxicity induced by CIS. Therefore, the findings of

the study should be supported by further clinical studies. Our experimental study results need to be supported by further clinical studies.

**Ethical approval:** The study protocol was approved by the Ethics Committee on Animal Research (reference no: 2015/A-84 – 22.10.2015) under the Faculty of Medicine, Inonu University, Malatya, Turkey.

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**Author Contributions:** KEB, OO, RB, AA, and HP are the coordinators of this study and they planned the study protocol design. KEB, MG, OO, RB, and HP made the mandatory requirements for the study. KEB and OO were responsible for drug administration. OO and MG were responsible for data collection. KEB, OO and HP performed the surgical procedures. YT conducted the biochemical analyses. The histopathological evaluations carried out by NV and AY whereas AY and MG performed statistical analysis. MG and HP were responsible for interpretation of the results. MG was responsible for the design of figures and tables. This manuscript was written by MG, OO, and HP. The final manuscript is revised collaboratively by MG and HP.

**Conflict of Interest:** The authors declare that there are no conflicts of interest.

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