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Secoisolariciresinol Diglucoside as a Potential Hepatoprotective Agent Against Mercuric Chloride-Induced Oxidative Damage

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Abstract

Mercuric chloride ($HgCl_2$)-induced hepatotoxicity involves oxidative stress and apoptosis, posing a significant public health concern. Secoisolariciresinol Diglucoside (SDG), a bioactive lignan derived from flaxseed, exhibits potent antioxidant and anti-inflammatory properties. This study investigated the hepatoprotective potential of SDG in female Wistar rats exposed to $HgCl_2$. Twenty-four rats were allocated into four groups: control, $HgCl_2$ (2 mg/kg, intraperitoneal), SDG (5 mg/kg, subcutaneous), and $HgCl_2$ + SDG (co-administered one hour apart). Biochemical analyses included oxidative stress markers (malondialdehyde, total oxidant status, oxidative stress index), antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), liver function tests (ALT, AST, ALP, bilirubin), and histopathological examination. Exposure to $HgCl_2$ significantly increased oxidative markers and liver enzymes while reducing antioxidant enzyme activities, indicating severe hepatic damage. Treatment with SDG significantly ameliorated these changes, restoring redox balance and preserving hepatic tissue architecture. These findings demonstrate that SDG confers hepatoprotection against $HgCl_2$-induced liver injury, likely through its antioxidant capacity and modulation of oxidative stress pathways, supporting its potential as a therapeutic agent for heavy metal-induced hepatotoxicity.

Keywords: Secoisolariciresinol Diglucoside (SDG), Mercuric Chloride, Hepatotoxicity, Oxidative stress, Antioxidant enzymes.

1. Introduction

Liver diseases represent a prominent global health challenge, with hepatotoxicity induced by heavy metals such as mercuric chloride ($HgCl_2$) standing as a substantial concern (Oriquat et al., 2012;Singh et al., 2024). Exposure to heavy metals precipitates a cascade of deleterious effects within hepatic tissues, primarily characterized by oxidative stress, inflammatory responses, and programmed cell death. Collectively, these mechanisms contribute to hepatic injury (Jomova, et al., 2024).

Mercury (Hg) is a shiny, silvery heavy metal known for its hazardous and toxic characteristics. It is widely used in cosmetics formulation and is prevalent in both industrial and environmental settings (Yang et al., 2020,Olomukoro et al., 2009). Originating mainly from industrial processes, mining, and natural sources, mercury is recognized as an environmental pollutant and neurotoxin (de Paula Arrifano et al., 2023). It contaminates various human food sources, including fish, seafood, grains, and vegetables (Uddin, Khanom, & Islam, 2023). Mercury exposure adversely affects multiple organs, notably the central nervous system, liver, and kidneys (Yang et al., 2020).

Acute liver injury, characterized by sudden impairment in liver function, results in the accumulation of metabolic waste products. Both acute and chronic liver pathologies involve degenerative changes in hepatic tissues, leading to liver failure (Sarin et al., 2019). The significant role of antioxidants in counteracting mercury toxicity, reducing its symptoms, and minimizing its accumulation in biological tissues (AlRamadneh et al., 2022; Unsal, Dalkıran, Çiçek, & Kölükçü, 2020).

Secoisolariciresinol Diglucoside (SDG), a bioactive lignan naturally present in flaxseed, has gained considerable scientific interest for its potent hepatoprotective effects (Noreen, Rehman, Tufail, Badar Ul Ain, & Awuchi, 2023). These protective properties are primarily attributed to its strong antioxidant capacity and anti-inflammatory mechanisms, which play a crucial role in mitigating hepatic oxidative damage and inflammation(Tse et al., 2023). Excessive production of reactive oxygen species (ROS) is a key contributor to the pathophysiology of various clinical disorders, driving oxidative stress and apoptosis-mediated cellular dysfunction. SDG, recognized for its potent antioxidant properties, exerts its protective effects by inhibiting NADPH oxidase (NOX), thereby limiting ROS generation (AlRamadneh et al., 2023; Osmakov, Kalinovskii,

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Belozerova, Andreev, & Kozlov, 2022). Through this mechanism, SDG is proposed to suppress neutrophil oxidative burst and chemotaxis, ultimately mitigating neutrophil-induced tissue injury and inflammatory damage (Mecocci et al., 2022).

This study aims to evaluate the hepatoprotective potential of SDG against chemically induced liver toxicity using a well-established rat model. By elucidating the biochemical, and histological, underlying SDG's protective effects, this research seeks to provide a comprehensive understanding of its therapeutic potential in mitigating heavy metal-induced hepatic damage and oxidative stress.

2. Materials and Methods

2.1. Chemicals and kits

Secoisolariciresinol Diglucoside (SDG) obtained from Dr.Sayed Mujahed Hashimi, (Griffith University, Australia)., Mercuric Chloride was purchased from Sigma.

TAC was purchased from Labor Diagnostika Nord Co., Germany. TOS values using a novel automated measurement method developed by Erel using colorimetric kits (Rel Assay, Türkiye). Serum liver function tests were purchased from Diagnostic Medical International, Diamond Jordan. GSH, GPX, CAT and SOD were purchased from Diagnostic Medical International, Diamond Jordan. The MDA kits were purchased from Nanjing Jiancheng Bioengineering Co. Ltd, China.

2.2. Animals

Animal experiments adhered to institutional guidelines (Approval No. ZU-2024/2/2). A total of 24 female Wistar Albino rats (4 months old, weighing 140–160 g) were selected for this study. The choice of female rats was made arbitrarily and did not influence the experimental outcomes. The rats were acquired from Yarmouk University. Throughout the study, each rat was fed a standard diet of rat pellets and tap water. The rats were housed under controlled environmental conditions, maintaining a temperature of 24 ± 5°C, 60% relative humidity, and a 12-hour light-dark cycle. Animals were randomly assigned to experimental groups to ensure unbiased distribution.

The rats were randomly assigned to four experimental groups (n = 6 per group). Group I (control) received an intraperitoneal (i.p.) saline injection. Group II was administered Mercuric Chloride ($HgCl_2$) at a dose of 2 mg/kg via i.p. injection. Group III received Secoisolariciresinol Diglucoside (SDG) at 5 mg/kg subcutaneously (S.Q.) once daily for seven days. Group IV was administered $HgCl_2$ (2 mg/kg, i.p.) daily for seven days, followed by an S.Q. injection of SDG (5 mg/kg) one hour after each $HgCl_2$ dose. (Figure 1)

Throughout the experiment, toxicity signs, body weight, and food intake were systematically monitored. All animals received their respective treatments at the same time each day. On day 8, the final body weights were recorded, and the animals were euthanized. Blood samples were collected via cardiac puncture, and liver tissues were promptly excised for biochemical and histological analyses.

Liver homogenates were prepared to assess oxidative stress biomarkers, including malondialdehyde (MDA) as a lipid peroxidation indicator, and key antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH). Additionally, total antioxidant capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were quantified to evaluate the overall redox balance. Histological examination of the remaining formaldehyde-preserved liver tissue was conducted using conventional light microscopy procedures.

2.3. Liver Function Tests

Serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin were quantified as markers of liver function using commercially available diagnostic kits (Randox Laboratory, Crumlin, UK).

2.4. Determination of antioxidant enzyme activities

Liver tissues were homogenized in 0.05 mol/L phosphate buffer and centrifuged at 3,500 rpm for 30 min at 4°C. The supernatant was collected for biochemical analyses, including lipid peroxidation and antioxidant enzyme activity assessments, using commercially available assay kits (Nanjing Jiancheng Bioengineering Co. Ltd, China).

Malondialdehyde (MDA) levels were quantified as an indicator of lipid peroxidation by measuring thiobarbituric acid-reactive substances (TBARS) at 532 nm, with results expressed as nmol/g protein. Superoxide dismutase (SOD) activity was determined based on the reduction of nitroblue tetrazolium (NBT) by superoxide anions generated through the xanthine/xanthine oxidase system. One unit of SOD activity was defined as the enzyme concentration required to inhibit NBT reduction by 50%, expressed as U/mg protein.

Catalase (CAT) activity was assessed by measuring the formation of a yellow complex between molybdate and H_2O_2 at 405 nm. Enzymatic activity was defined as the degradation of 1 μmol H_2O_2 per second per mg of tissue protein, with results expressed as kcal/g protein. Glutathione peroxidase (GPx) activity was evaluated by monitoring absorbance changes at 340 nm following the addition of H_2O_2 to a reaction mixture containing glutathione reductase, reduced glutathione, sodium azide, and NADPH. GPx activity was expressed as U/g protein.

All assays were performed according to the manufacturer's protocol. Total protein content was determined using the Lowry method, with bovine serum albumin as the standard.

2.5. Determination of total antioxidant capacity (TAC) in Rats Serum

Total antioxidant capacity (TAC) was measured according to the method of (Koracevic, Koracevic, Djordjevic, Andrejevic, & Cosic, 2001), using EIA kit that was purchased from Labor Diagnostika Nord Co., Germany.

2.6. Determination of Total Oxidant Status (TOS) in Rats Serum

Total oxidant status (TOS) was measured in serum using a novel automated colorimetric method developed by Erel, employing commercial assay kits (Rel Assay, Türkiye). This method is based on the oxidation of ferrous iron-O-dianisidine complexes to ferric ions by sample

oxidants. The oxidation process is facilitated by glycerol molecules, which may be abundant in the reaction medium.

In an acidic environment, the generated ferric ions react with xylenol orange, forming a highly colored complex. The intensity of this color change was quantified spectrophotometrically, providing a measure of the total oxidant molecules in the sample. TOS levels were expressed as millimoles per liter (mmol) of H_2O_2 equivalent, with hydrogen peroxide serving as the reference standard.

2.7. Determination of Oxidative Stress Index (OSI) in Rats Serum

Oxidative stress was quantified using the Oxidative Stress Index (OSI), which represents the balance between total oxidant status (TOS) and total antioxidant capacity (TAC). OSI was calculated using the formula: OSI (arbitrary units) = TOS / TAC. The same method was applied to determine OSI values in liver tissue samples, providing a comprehensive assessment of oxidative stress levels.

2.8. Histopathological Methods

Liver tissue samples were fixed in 10% neutral-buffered formalin for 48 hours to preserve structural integrity. The samples were then dehydrated through a graded series of ethanol concentrations to ensure optimal tissue processing for histological analysis. Subsequently, the samples were embedded in paraffin for further processing. Using a microtome, 4 mm thick sections were cut from the paraffin blocks.

Histopathological examination was conducted using hematoxylin and eosin (H&E) staining to visualize tissue architecture and cellular morphology. Specifically, H&E staining allows for the differentiation of cell nuclei (stained blue by hematoxylin) and cytoplasmic structures (stained pink by eosin).

A pathologist, blinded to the treatment groups, examined the stained tissue sections under a light microscope (Nikon Eclipse, E600 W. Tokyo, Japan) to assess for any histological change's indicative of liver damage or pathology. Images of the tissue sections were captured using a digital camera (Nikon Microscope Digital Camera DP70, Tokyo, Japan) for documentation and analysis.

This meticulous histopathological analysis enabled the identification and characterization of any structural alterations or abnormalities in the liver tissue, providing valuable insights into the effects of the experimental treatments on liver health and function.

2.9. Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). Statistical differences among the groups were analyzed using one-way ANOVA. Tukey's post hoc test ($p < 0.05$) was employed to determine the significance of the differences. GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA) was used for all statistical analyses in this study.

3. Results

3.1. Body Weight

Animal body weights were recorded daily throughout the experimental period. Notably, the group exposed to $HgCl_2$ exhibited a significant reduction in body weight compared to the other groups, suggesting an adverse effect of $HgCl_2$ on metabolic homeostasis, indicating a decline in body weight over the course of the experiment. This decrease in body weight gain suggests that exposure to $HgCl_2$ resulted in either growth inhibition or weight loss in the rats (Table 1).

However, intriguingly, the group treated with both $HgCl_2$ and SDG demonstrated a partial restoration of body weight gain compared to the $HgCl_2$ group. This observation suggests a potential protective effect of SDG against the adverse effects of $HgCl_2$ on body weight.

These findings underscore the potential of SDG as a hepatoprotective in mitigating the detrimental effects of heavy metal exposure on physiological parameters such as body weight. Further investigation is warranted to elucidate the underlying mechanisms responsible for the observed protective effects of SDG and its potential implications for human health.

Table 1. Effect of Secoisolariciresinol Diglucoside (SDG) on body weight of rats that received Mercuric Chloride.

Parameters*	Control	SDG	Mercuric Chloride	Mercuric Chloride + SDG
Initial body weight (g)	155.27±7.28	153.17± 6.73	159.48±6.89	148.87±7.44
Final body weight (g)	167.16±8.89	162.18±8.96	152.76±10.37[a]	153.44±9.48[b]
Body weight gain (g)	11.09±6.15	9.01±6.22	-6.72±4.13[a]	4.57±2.19[b]

Data are presented as mean ± SEM. [a] Indicates a statistically significant difference compared to the control group ($p < 0.05$). [b] Indicates a statistically significant difference compared to the Mercuric Chloride-treated group ($p < 0.05$).

3.2. Liver Function Tests

The results revealed a significant increase in ALP, AST, and ALT levels in the group treated solely with $HgCl_2$ compared to the control group. This elevation suggests hepatocellular damage induced by $HgCl_2$. However, intriguingly, the group receiving both $HgCl_2$ and SDG exhibited significantly lower levels of ALP, AST, and ALT compared to the $HgCl_2$ -only group (Table 2).

Additionally, there was a significant difference in bilirubin levels between the control group, the Secoisolariciresinol Diglucoside (SDG)-treated group, and the group treated with both $HgCl_2$ and SDG. A noticeable increase in bilirubin levels was observed in the group treated solely with $HgCl_2$ compared to the control group.

These findings suggest a potential protective effect of SDG against $HgCl_2$ -induced hepatocellular damage, as evidenced by the significant reduction in ALP, AST, and ALT levels in the co-treated group. However, further investigation is required to elucidate the underlying mechanisms responsible for these observed effects and their potential clinical implications.

Table2. Effects of Secoisolariciresinol Diglucoside (SDG) on serum parameters in rats treated with Mercuric Chloride.

Parameters*	Control	SDG	Mercuric Chloride	Mercuric Chloride + SDG
ALP (IU/L)	36.76±7.13	34.72±7.94	65.03±13.56[a]	41.34±14.10[b]
AST(IU/L)	85.47±9.18	84.71±10.34	148.64±13.13[a]	95.85±11.93[b]
ALT (IU/L)	71.18±6.09	70.49±8.34	129.30±14.49[a]	82.76±14.08[b]
Bilirubin (Um/l)	1.62±0.24	1.41±0.24	3.07±0.9[a]	1.9±0.55[b]

Data are presented as mean ± SEM. [a] Indicates a statistically significant difference compared to the control group ($p < 0.05$). [b] Indicates a statistically significant difference compared to the Mercuric Chloride-treated group ($p < 0.05$).

3.3. Oxidant – Antioxidant Status.

The effects of SDG on Oxidant and Antioxidant Status in liver homogenate rats were investigated to evaluate its hepatoprotective properties. The Antioxidant enzyme included SOD, GPX, CAT levels, MDA, TOS, TAC, GSH levels, and OSI.

According to Table 3, the results show a significant decrease in SOD, GPX, and CAT activity in the liver homogenate of rats treated with $HgCl_2$ alone compared to the control group, indicating a depletion of antioxidant defenses. However, the group treated with both $HgCl_2$ and SDG exhibited significantly higher SOD activity compared to the $HgCl_2$ -only group.

Additionally, the results reveal a significant increase in MDA levels in the group treated with $HgCl_2$ alone compared to the control group, indicating enhanced lipid peroxidation. However, supplementation with SDG significantly reduced MDA levels compared to the mercuric chloride-only group (Figure 2).

According to Figure 3-a, the results demonstrated a significant increase in TOS levels in rats treated with $HgCl_2$ alone compared to the control group. This elevation indicates an augmentation of oxidative stress due to $HgCl_2$ exposure. However, when SDG was administered concurrently with mercuric chloride, there was a notable decrease in TOS levels compared to the mercuric chloride-only group. Interestingly, according to Figure 3-b there were no significant differences observed in TAC levels among the experimental groups. This indicates that neither mercuric chloride nor SDG treatment significantly altered the overall antioxidant capacity of the liver tissue in this study.

Furthermore, according to Figure 3-c, the results also showed a significant increase in OSI in rats treated with mercuric chloride alone, indicating a shift towards oxidative stress. Conversely, administration of SDG alongside mercuric chloride resulted in a significant reduction in OSI compared to the mercuric chloride-only group.

Finally, according to Figure 4, the results revealed a significant depletion of GSH levels in rats treated with $HgCl_2$ alone compared to the control group, suggesting impaired antioxidant defense mechanisms. However, supplementation with SDG led to a significant restoration of GSH levels compared to the $HgCl_2$ -only group.

Table 3. Impact of Secoisolariciresinol Diglucoside (SDG) on theAntioxidants in Mercuric Chloride–Treated Liver Homogenate

Parameters*	Control	SDG	Mercuric Chloride	Mercuric Chloride + SDG
SOD(U/mg protein)	1.77±0.21	1.79±0.31	0.84±0.23[a]	1.23±0.27[b]
GP_X (U/mg protein)	173.25±12.76	170.02±16.19	117.64±17.46[a]	157.86±17.23[b]
CAT (k/g protein)	31.47±6.23	29.15±7.13	17.32±5.90[a]	24.95±6.17[b]

Data are presented as mean ± SEM. [a] Indicates a statistically significant difference compared to the control group ($p < 0.05$). [b] Indicates a statistically significant difference compared to the Mercuric Chloride-treated group ($p < 0.05$).

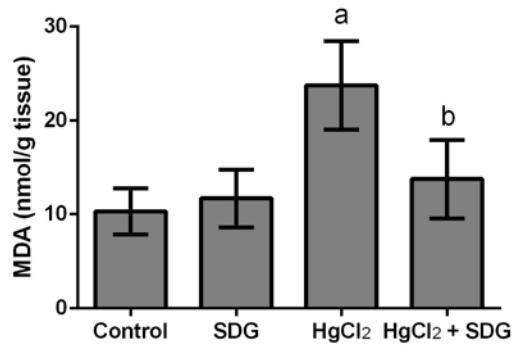

Figure 2. Impact of Secoisolariciresinol Diglucoside (SDG) on MDA in Mercuric Chloride -treated liver homogenate. Data are presented as mean ± SEM. [a] Indicates a statistically significant difference compared to the control group ($p < 0.05$). [b] Indicates a statistically significant difference compared to the Mercuric Chloride-treated group ($p < 0.05$).

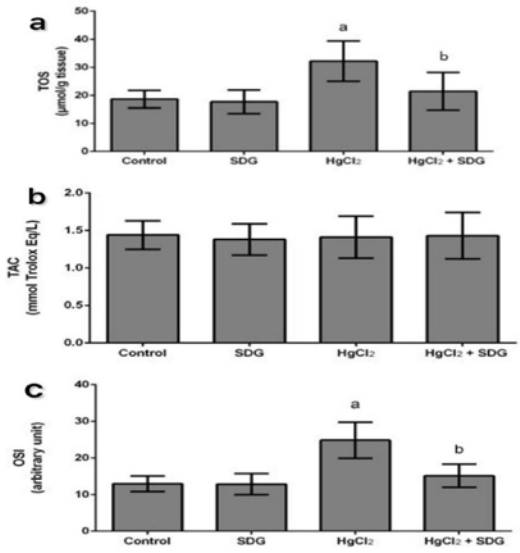

Figure 3. Effects of Secoisolariciresinol Diglucoside (SDG) on the Serum parameters TOS, TAC and OSI for rats received Mercuric Chloride. Data are presented as mean ± SEM. [a] Indicates a statistically significant difference compared to the control group ($p < 0.05$). [b] Indicates a statistically significant difference compared to the Mercuric Chloride-treated group ($p < 0.05$).

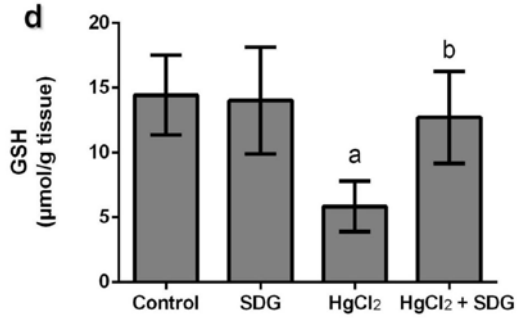

Figure 4 . Effects of Secoisolariciresinol Diglucoside (SDG) on the Serum GST for rats received Mercuric Chloride. Data are presented as mean ± SEM. a Indicates a statistically significant difference compared to the control group ($p < 0.05$). b Indicates a statistically significant difference compared to the Mercuric Chloride-treated group ($p < 0.05$).

3.4. Histopathological Examination

The histopathological examination of liver tissues showed clear group-specific differences. The control group exhibited normal hepatic architecture, with radially arranged hepatocytes around a central vein and intact sinusoidal spaces (Figure 5-A). Similarly, the SDG-treated group maintained a preserved lobular structure with no signs of inflammation or vascular damage, indicating the absence of hepatic injury (Figure5-B). In contrast, the group exposed to $HgCl_2$ alone displayed pronounced pathological alterations, including congested central veins, dilated sinusoids, cytoplasmic vacuolization in hepatocytes, and scattered inflammatory infiltration—hallmarks of oxidative stress-induced hepatotoxicity (Figure 5-C). Notably, co-treatment with SDG and $HgCl_2$ resulted in substantial histological improvement. The hepatic parenchyma appeared mostly intact, with near-normal cellular organization and reduced signs of congestion or vacuolation (Figure 5-D), suggesting that SDG exerts a protective effect against $HgCl_2$-induced liver damage by preserving histological architecture and mitigating tissue injury.

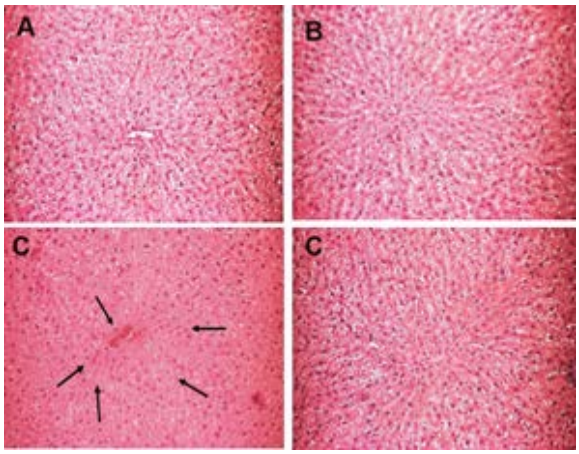

Figure 5. Histology of rat liver after (Mercuric Chloride (HgCl2) and Secoisolariciresinol Diglucoside (SDG) treatment for five days.

a) Control group, showing normal morphology.
b) Secoisolariciresinol Diglucoside (SDG) group, showing normal morphology.
c) Mercuric Chloride (HgCl2) group,The liver section reveals vascular congestion with evidence of hepatocellular degeneration, including cytoplasmic vacuolation and pyknotic nuclei. Loss of classical hepatic cord arrangement and dilated sinusoids .
d) Mercuric Chloride (HgCl2) + Secoisolariciresinol Diglucoside (SDG) group. Near normal histology of liver cells compared to the control group.

4. Discussion

Despite extensive research on the systemic toxicity of $HgCl_2$ at high doses, its precise impact on hepatic health remains insufficiently characterised. Accumulating evidence suggests that oxidative stress plays a central role in $HgCl_2$-induced hepatotoxicity by triggering apoptotic pathways and disrupting cellular homeostasis. The generation of reactive oxygen species (ROS), primarily mediated by NADPH oxidase (NOX), has been identified

as a key contributor to oxidative damage and hepatocellular apoptosis.

ROS overproduction leads to oxidative modifications of lipids, proteins, and DNA, resulting in mitochondrial dysfunction, endoplasmic reticulum stress, and activation of pro-apoptotic signalling cascades (Bhatti, Bhatti, & Reddy, 2017; Juan, Pérez de la Lastra, Plou, & Pérez-Lebeña, 2021). This oxidative imbalance compromises antioxidant defence mechanisms, thereby exacerbating liver injury (Videla & Valenzuela, 2022). Understanding the molecular interplay between $HgCl_2$ exposure, NOX activation, and apoptotic pathways is critical for developing targeted hepatoprotective strategies.

This study evaluated the hepatoprotective potential of Secoisolariciresinol Diglucoside (SDG) against $HgCl_2$-induced liver toxicity. Recognized for its potent antioxidant capacity and free radical-scavenging properties, SDG was investigated for its ability to counteract oxidative stress and apoptotic mechanisms associated with $HgCl_2$ exposure. Notably, our findings indicate that the administration of $HgCl_2$ at a dosage of 2 milligrams per kilogram resulted in hepatotoxicity, as evidenced by histopathological alterations, elevated liver oxidative stress markers, and serum ALT, AST, ALP and Bilirubin levels. Importantly, these alterations were validated through experimental studies.

Biochemical analyses revealed a significant increase in MDA formation in liver tissue extracts from individuals treated with $HgCl_2$ compared to the control group. Conversely, the $HgCl_2$ + SDG group exhibited lower signs of oxidative stress. These findings suggest that $HgCl_2$ induces oxidative stress, leading to heightened MDA production, a lipid peroxidation product. Consequently, this process contributes to the development of hepatotoxicity.

The significant reduction in the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) induced by $HgCl_2$ underscores its impact on the antioxidant defense mechanisms. In contrast, the group receiving SDG exhibited a pronounced increase in these enzyme activities compared to the control group.

Hepatocytes possess both enzymatic and non-enzymatic antioxidant systems to maintain cellular membrane stability under oxidative stress conditions (Mirończuk-Chodakowska, Witkowska, & Zujko, 2018; Santovito et al., 2021). Dysregulation of these systems can lead to various diseases. These hepatocytes' endogenous enzymatic antioxidants, including SOD, CAT, and GPx, play crucial roles in neutralizing free oxygen radicals (He et al., 2017). SOD functions by converting superoxide radicals into hydrogen peroxide, while CAT and GPx further decompose hydrogen peroxide into water and oxygen (Rai, Sonne, & Kim, 2023).

Moreover, GPx operates via the glutathione (GSH) reduction pathway, with GSH being regenerated by glutathione reductase (Flohé, Toppo, & Orian, 2022). Elevated enzyme levels serve as a protective response to oxidative stress, shielding tissues from damage (Blount, Vitikainen, Stott, & Cant, 2016). SDG, acting as a potent NADPH oxidase (NOX) inhibitor, mitigates oxidative stress, potentially through increased enzyme levels (AlRamadneh et al., 2023).

It is important to note that superoxide generation is not solely mediated by NADPH oxidase (NOX); alternative enzymatic pathways, including xanthine oxidase, glutathione S-transferase, and cytochrome P450, also contribute to ROS production (L. Zhang et al., 2019). SDG appears to exert a broader regulatory effect on oxidative stress pathways, modulating catalase (CAT) activity and initiating a compensatory antioxidant response (Aqeel, Gurumallu, Bhaskar, & Javaraiah, 2020; Newsholme, Keane, Carlessi, & Cruzat, 2019). This includes the upregulation of glutathione peroxidase (GPx) synthesis, particularly under conditions where superoxide dismutase (SOD) and CAT activity are compromised. These findings suggest that SDG may play a multifaceted role in maintaining redox homeostasis by influencing multiple antioxidant defense mechanisms (Rai, Sonne, & Kim, 2023).

Our findings support the established concept that excessive ROS generation disrupts antioxidant defence mechanisms, thereby driving hepatotoxicity. The imbalance between oxidative stress and antioxidant capacity contributes to lipid peroxidation, protein oxidation, and mitochondrial dysfunction, ultimately exacerbating liver injury. These results further emphasize the critical role of oxidative stress in mediating $HgCl_2$-induced hepatocellular damage and highlight the importance of antioxidant interventions in mitigating hepatic toxicity (AlRamadneh et al., 2022). Consistent with prior studies, $HgCl_2$-induced hepatotoxicity is marked by a significant elevation in liver enzyme levels, indicative of hepatocellular injury, alongside a dysregulated antioxidant defense system. This disruption is primarily driven by excessive lipid peroxidation and protein oxidation, which contribute to oxidative stress, mitochondrial dysfunction, and inflammatory responses. These findings further reinforce the role of oxidative damage in the pathophysiology of $HgCl_2$-induced liver toxicity (Karuppanan, Krishnan, Padarthi, & Namasivayam, 2014; Oriabure & Innih, 2024; Raeeszadeh, Moradi, Ayar, & Akbari, 2021)

The glutathione (GSH) cycle represents a fundamental intracellular antioxidant defense system, playing a critical role in maintaining redox balance and mitigating oxidative stress (Liu, Sun, Zhang, Wang, & Zheng, 2022). This cycle is central to cellular detoxification processes, facilitating the neutralization of reactive oxygen species (ROS) and lipid peroxides through the coordinated activity of glutathione peroxidase (GPx) and glutathione reductase (Scirè et al., 2019). Numerous antioxidant enzymes rely on GSH as a substrate for their function, with GPx being a key enzyme that is directly dependent on intracellular glutathione levels (Vašková, Kočan, Vaško, & Perjési, 2023). Adequate GSH availability is essential for GPx-mediated detoxification of hydrogen peroxide (H_2O_2), catalyzing its conversion into water and molecular oxygen, thereby preventing oxidative damage and preserving hepatocellular integrity (Torquato, Principato, Galli, & Armeni, 2018). This process leads to the formation of glutathione disulfide (GSSG) when reduced glutathione (GSH) is oxidized by glutathione reductase (Baba & Bhatnagar, 2018). Reduced GPx activity results in the accumulation of toxins, exacerbating oxidative stress(Ribeiro et al., 2023). Our research findings indicate that $HgCl_2$ significantly reduces both GPx and GSH levels, consistent with previous studies.

Furthermore, our study demonstrated a significant increase in total oxidant status (TOS) and oxidative stress index (OSI), accompanied by a reduction in total antioxidant capacity (TAC) in the $HgCl_2$ treated group compared to the $HgCl_2$ + SDG co-treatment group. Notably, while SDG supplementation effectively reduced TOS and OSI levels, no significant alterations were observed in TAC. These findings indicate that $HgCl_2$ exposure induces a pronounced oxidative stress response, which is partially attenuated by SDG, likely through its antioxidative and free radical-scavenging properties. Liver function tests serve as a straightforward method to assess hepatotoxicity (Soldatow, LeCluyse, Griffith, & Rusyn, 2013). Elevated serum alanine aminotransferase (ALT) levels are indicative of hepatotoxicity and cell damage (McGill, 2016). Consistent with this, the $HgCl_2$ group exhibited increased ALT levels, whereas the SDG group showed decreased ALT levels. This further supports the antioxidant properties of SDG, as evidenced by its ability to reduce oxidative stress.

Light microscopy was performed to examine the morphological alterations in the liver. Histopathology studies revealed that the $HgCl_2$ group had vasoconstriction and thrombosis in the central veins, pericentre and periportal sinusoidal dilatation, parenchymal inflammation, vacuole alterations in hepatocytes, and biliary channel growth. The inclusion of SDG into the $HgCl_2$ regimen resulted in a reduction of these alterations. According to previous studies, SDG resulted in a dramatic reduction in ROS and apoptosis via blocking NOX (AlRamadneh et al., 2023; Z. Zhang et al., 2023). Similar to these findings, ours confirms the anti-apoptotic action of SDG. MDA, TOS, OSI, and serum ALT levels, together with decreased SOD, CAT, and GPx contents, were seen in the $HgCl_2$ group, but the same parameters were reduced in the $HgCl_2$ + SDG group, indicating that the histological alterations were consistent with the biochemical findings.

Histopathological analysis using light microscopy revealed significant morphological alterations in the livers of rats exposed to $HgCl_2$. These changes included vasoconstriction and thrombosis in central veins, sinusoidal dilation in both pericentral and periportal regions, parenchymal inflammation, hepatocyte vacuolization, and biliary channel proliferation.

Notably, co-administration of SDG with $HgCl_2$ markedly attenuated these histopathological changes, suggesting a protective effect. Previous studies have demonstrated that SDG exerts its hepatoprotective properties by mitigating oxidative stress and apoptosis, primarily through the inhibition of NADPH oxidase (NOX), a key enzymatic source of reactive oxygen species (ROS) (AlRamadneh et al., 2023; Osmakov et al., 2022; Zhu et al., 2022). Our findings align with previous studies, further confirming the anti-apoptotic properties of Secoisolariciresinol Diglucoside (SDG). Notably, rats exposed to $HgCl_2$ ($HgCl_2$) exhibited elevated levels of malondialdehyde (MDA), total oxidant status (TOS), oxidative stress index (OSI), and serum alanine aminotransferase (ALT), alongside significant reductions in the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

However, co-administration of SDG with $HgCl_2$ significantly attenuated these oxidative and biochemical alterations, restoring antioxidant enzyme activity while reducing oxidative damage markers. These biochemical improvements were consistent with the observed histological findings, reinforcing the hepatoprotective role of SDG.

Nephrotoxicity, neurotoxicity, and ototoxicity are well-documented side effects associated with exposure to $HgCl_2$ (Aqeel et al., 2019). Previous studies have linked the elevation of reactive oxygen species (ROS) to the development of Mercuric Chloride-induced ototoxicity and nephrotoxicity (Caglayan, Kandemir, Yildirim, Kucukler, & Eser, 2019; Kandemir, Yildirim, Caglayan, Kucukler, & Eser, 2019; Kang, Wang, Guo, & Yang, 2024). Specifically, NADPH oxidase, a crucial component in $HgCl_2$ toxicity, has been implicated in ROS production (Ahmad & Mahmood, 2019; AlRamadneh et al., 2023). It is widely recognized that $HgCl_2$ disrupts the body's enzymatic and non-enzymatic antioxidant systems, including glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase, while concurrently increasing oxidant systems such as malondialdehyde (MDA) and toxic lipid peroxides (Tyagi, Kalia, Chundawat, & Sood, 2018).

Numerous therapeutic agents, including SDG, have been investigated for their potential to alleviate hearing loss and kidney damage induced by $HgCl_2$ (AlRamadneh et al., 2022; Cappelletti et al., 2019). These properties suggest that SDG may hold promise as a protective agent against the adverse effects of $HgCl_2$ on hearing and kidney function (AlRamadneh et al., 2023).

Across the studies reviewed, researchers consistently concluded that the positive effects of SDG may be attributed to its antioxidant properties. By suppressing NADPH oxidase, SDG functions as a free radical scavenger, thereby mitigating oxidative stress. Despite limitations in resources, our study aimed to assess the most critical oxidative and antioxidant parameters. However, this restricted our ability to evaluate other oxidative stress markers such as conjugated dienes, lipid hydroperoxides, protein carbonylation, and total sulfhydryl concentration in the liver. Further research incorporating these additional markers could provide a more comprehensive understanding of the effects of SDG on oxidative stress and liver function.

In conclusion, free radicals and oxidative stress are pivotal factors in Mercuric Chloride-induced hepatotoxicity, with intricate underlying processes. Given their adverse impact on biological systems, it is imperative to effectively regulate and manipulate the production and activity of reactive oxygen species. Our current research has shown that SDG may alleviate the histopathological and biochemical manifestations of Mercuric Chloride-induced hepatotoxicity through its antioxidant and free radical scavenging properties.

We hypothesize that SDG could prevent Mercuric Chloride-induced liver damage. However, further clinical studies are warranted to explore the role of SDG in protecting the liver from the detrimental effects of Mercuric Chloride. Additional research in this area would enhance our understanding and provide valuable insights into the therapeutic potential of SDG in combating Mercuric Chloride-induced hepatotoxicity.

5. Conclusion

In conclusion, this study highlights the hepatoprotective properties of Secoisolariciresinol Diglucoside (SDG) against Mercuric Chloride-induced oxidative stress and apoptosis. Both biochemical and histological assessments validate the protective effects of SDG against hepatotoxicity by modulating antioxidant enzyme activity and decreasing oxidative stress indicators. These findings suggest that SDG holds great promise as a potential therapeutic agent for liver protection against toxic insults. Future research should explore its molecular mechanisms and potential clinical applications to reinforce its efficacy in preventing chemically induced hepatic damage.

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