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Experimental Research Article

Alpha lipoic acid and *Cleome droserifolia* extract as possible protective agents against mercuric chloride-induced hepatorenal toxicity in male albino rats

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ABSTRACT

Introduction: Mercury chloride is commonly used in our daily lives due to its diverse applications and can induce hepatorenal toxicity even at low doses. The present investigation studies the preventive effects of lipoic acid and *Cleome droserifolia* extract against mercury-induced hepatorenal toxicity.

Methods: Thirty male albino rats were randomly assigned to six experimental groups. The first group served as the normal control. The second group was treated with alpha-lipoic acid (ALA) at a dose of 10 mg/kg. The third group received Cleome droserifolia extract (CD) at the same dose. The fourth group was exposed to mercuric chloride ($HgCl_2$) at 35 mg/kg (equivalent to 21% of the LD_{50}). The fifth group was co-treated with alpha-lipoic acid (10 mg/kg) and mercuric chloride (35 mg/kg), while the sixth group received *Cleome droserifolia* extract (10 mg/kg) together with mercuric chloride (35 mg/kg). All treatments were administered orally once daily for a period of eight weeks.

Results: Mercury induced a slight decline in body weights and relative organ weights for the liver and kidney compared to the normal control group. It caused significant elevations ($p<0.05$) in hamoglobin concentration and white blood cell (WBC) count; however, bone marrow cell count was not affected. Mercury triggered considerable disruption in liver and kidney functions. It also promoted a significant decline in catalase (CAT) activity and a significant elevation in malondialdehyde (MDA) levels. Mercury-induced degeneration, fibrosis, and necrosis in the liver and kidney tissues. Administration of alpha lipoic acid and *Cleome droserifolia* extract showed marked improvement in the different parameters under investigation.

Conclusion: Lipoic acid was found to be more effective against mercury chloride-induced hepatorenal toxicity than *Cleome droserifolia* extract.

Keywords:
Mercuric chloride
Lipoic acid
Cleome droserifolia extract
Fibrosis
Necrosis

Introduction

Mercury chloride ($HgCl_2$) is widely used in disinfectants, antiseptics, electronics, pigments, chemicals, and textile manufacturing. It is considered one of the

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most toxic forms of mercury because it can easily pass through biological membranes and form organomercury complexes with proteins (Joshi et al., 2017). Numerous toxicological and biological studies have reported that $HgCl_2$ causes neural (Abdel Moneim, 2015), hepatic (Joshi et al., 2014), nephrotic (Caglayan et al., 2019), and hematologic disturbances (Uzunhisarcikli et al., 2016) in experimental animals. The mechanism of $HgCl_2$ toxicity is related to the production of high amounts of reactive oxygen species (ROS), resulting in oxidative stress and depletion of glutathione and thiols (Hussain et al., 1997; Rao and Purohit, 2011). Evidence suggests that alpha-lipoic acid (ALA) is naturally present in most prokaryotic and eukaryotic organisms (Packer et al., 1997). ALA is produced by plants (Goraca et al., 2011), animals, and humans (Carreau, 1979). Alpha-lipoic acid is a lipid-soluble antioxidant (Packer et al., 1995).

Alpha-lipoic acid (ALA) acts as a coenzyme in the mitochondria, facilitating the activity of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase enzymes (Evans and Goldfine, 2000). It combats oxygen radicals and is renowned for capturing the radicals of hydroxyl and nitric oxide, as well as peroxynitrite and hydrogen peroxide anions, and for producing single oxygen atoms (Wong et al., 2001; Shay et al., 2009). In both its oxidized (ALA) and reduced (DHLA) forms, it serves as a potent antioxidant, demonstrating activity in both fat- and water-soluble environments (Goraca et al., 2011). Alpha-lipoic acid lowers reactive oxygen species (ROS) levels and regenerates the activity of other antioxidants (Sudheesh et al., 2013).

Samoa (*Cleome droserifolia*), a member of the Cleomaceae family, is commonly grown in various areas of North Sinai, Egypt. *Cleome droserifolia* holds medicinal and ecological significance. It is used in folk medicine for its digestive properties, as a rubefacient, and in the treatment of scabies, rheumatic fever, and inflammation (El-Shenawy and Abd El Nabi, 2006). It also possesses anticancer and hepatoprotective properties (Abdel-Kader et al., 2009). Samoa is used in the therapy of diabetes mellitus (El-Askary, 2005). These effects are attributed to its antimicrobial, analgesic, antipyretic, antioxidant, anti-inflammatory, and anti-schistosomiasis properties (Muhaidat et al., 2015; Sarhan et al., 2016; Ndamba et al., 1994). *Cleome droserifolia* enhances lipid metabolism, combats obesity, and enhances antioxidant activity in diabetic rats and mice (Marles, 1995). Studies on the beneficial effects of lipoic acid and *Cleome droserifolia* against mercuric chloride-induced toxicity have been limited. Therefore, this study aims to investigate the protective efficacy of lipoic acid and *Cleome droserifolia* extract against mercury-induced hepatorenal toxicity.

Materials and Methods

Chemicals

Mercuric chloride ($HgCl_2$, 99% purity) and alpha lipoic acid (1,2-Dithiolane-3-pentanoic acid, 6,8-Dithiooctanoic acid, DL-α-Lipoic acid, DL-6,8-Thioctic acid, Lip(S_2); $C_8H_{14}O_2S_2$, ≥98% purity) were purchased from Sigma-Aldrich, Germany.

Animals

Thirty (30) male albino rats (*Rattus norvegicus*), 10 weeks old and weighing 150 ± 25 g, purchased from the animal house at Suez Canal University, Egypt, were used as experimental animals. Animals were housed in plastic cages in six groups during the study.

Animals were maintained on a 12-hour light-dark cycle at a controlled temperature of 25 ± 2 °C and a relative humidity of 45 ± 5%. Animals were fed a standard, balanced diet and water and acclimatized for 15 days to the lab conditions. The protocol for the animal experimentation was approved by the Research Ethics Committee of the Faculty of Science, Arish University, with approval ID "ARU/SF.09." Our experimental procedures follow the guidelines for the care and use of laboratory animals, 8th ed., with strict adherence to ethical guidelines that comply with the ARRIVE guidelines.

Preparation of plant extract

The raw material of *Cleome droserifolia* (Forssk.) Delile, Cleo-maceae, was collected from Arish, North Sinai, Egypt, according to local and national rules and regulations. The plant material was recognized and taxonomized in the Faculty of Science at Arish University. Plant leaves were dried. To prepare *Cleome droserifolia* extract, 1 g of dried plant leaves was added to 100 ml of deionized water, stirred, then autoclaved at 121 °C for 20 min. After cooling, the mixture was filtered (Yang et al., 2019). The filtered extract was transferred to freeze-drying trays. The trays were frozen overnight at -80 °C, then inserted into a Freeze dryer (Labconco FreeZone freeze dryer, United States) for 72 hours at −50°C at 0.500 mbar to remove moisture, according to

(Phing et al., 2022). The dried material was weighed, placed into flakes, and milled into powder using a food processor (Panasonic Smart Food Processor MK-F800SSL, Japan). The dried powder extract was stored in an airtight container, away from light and moisture, until further use.

Experimental design

The animals were randomly subdivided into six groups of five rats. The first group served as the normal control. The second group received orally 10 mg/kg/day of alpha-lipoic acid (ALA group) (Adikwu et al., 2019). The third group received orally 10 mg/kg/day of *Cleome droserifolia* extract (CD group) (Al-Yahya, 2020). The fourth group received 35 mg/kg/day (21% of LD_{50}) of mercuric chloride ($HgCl_2$ group) (Clarkson, 2001). The fifth group received orally 10 mg/kg/day of alpha lipoic acid and 35 mg/kg/day of mercuric chloride (ALA + $HgCl_2$). The sixth group received orally 10 mg/kg/day of *Cleome droserifolia* extract and 35 mg/kg/day of mercuric chloride (CD+$HgCl_2$).

Duration time

After 8 weeks of treatment, animals were anesthetized using intramuscular injection of 50 mg/kg of ketamine, then rapidly dissected and tissues processed.

Collection of plasma samples

Blood samples were collected from the abdominal vein in EDTA tubes, then centrifuged for 15 minutes at 1000 *g* and stored at -30 °C until further biochemical analysis.

Hematological indices

Hematological measurements (hemoglobin and total white blood cell (WBC) count were conducted using a Diagon Ltd. D-Cell 60 fully automatic hematological analyzer.

Bone marrow collection and count

The right femur was cut from both ends, and bone marrow was washed with 2 mL of 0.9% NaCl in a tube. A 0.1 mL of bone marrow suspension was mixed with 0.9 mL of 0.2% eosin stain solution. Total bone marrow cells were counted on a hemocytometer using the method described by Dacic and Lewis (1991).

Preparation of tissues for microscopical examination

After animal dissection, the liver and kidney were immediately excised, blotted on filter paper, and weighed. Each organ was divided into two parts; the first part was kept in a 10% formalin solution for histological studies. The second part was stored at -30 °C.

Histopathology techniques

Five-micron-thick histological sections were stained with hematoxylin and eosin. The specimens were analyzed microscopically in a blinded manner (Edrissi, 2022).

Biochemical analysis

Parameters indicating oxidative stress were measured using biodiagnostic kits in Egypt. Catalase (CAT) activity was measured using the Aebi (1984) method. The malondialdehyde (MDA) concentration was determined using the technique of Ohkawa et al. (1979). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using a modified method of Schumann and Klauke (2003). Uric acid was measured utilizing Spinreact kits in Spain using the technique of Fossati et al. (1980). Creatinine was quantified using Diamond Diagnostics kits, Holliston, USA, by the Heinegard and Tiderstrom (1973) technique. Mercury concentration was determined by spectrometry in blood plasma using the method of Kopp et al. 1972.

Statistical analysis

The results represent the means ± standard deviation (SD) for five rats in each group. Data from the control and treated groups were analyzed using a one-way analysis of variance (ANOVA). The Tukey test was used for multiple comparisons. The difference was considered significant at $p < 0.05$. Statistical Package for Social Sciences (SPSS) software for Windows version 22.0 was used for statistical analysis.

Results

Body, liver, and kidney weights

Treatment with Mercury for eight weeks induced a slight decrease in body and relative organ weights (Figure 1A). However, insignificant increases in body and relative organ weights were observed in the ALA, CD, ALA & $HgCl_2$, and CD & $HgCl_2$ groups (Figure 1B, C) after eight weeks of treatment.

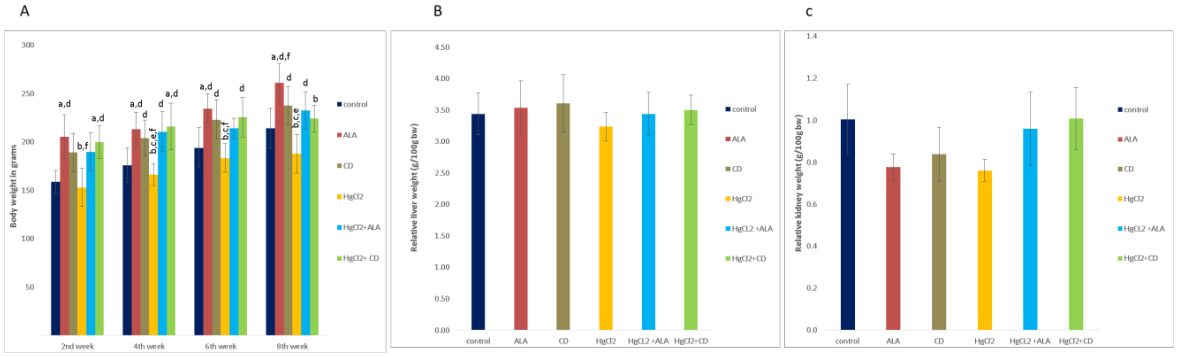

FIGURE 1. Effects of alpha-lipoic acid and Cleome droserfolia against mercuric chloride-induced effects on body weight (A), relative liver weight (g/100 g bw) (B), and relative kidney weight (g/100 g bw) (C) of rats. HgCl2: mercuric chloride; ALA: alpha lipoic acid; CD: Cleome droserfolia; bw: body weight. Data are expressed as mean ± SD for five rats per group. a: Significantly different from the control group ($p < 0.05$, Tukey's post hoc test). b: Significantly different from the alpha-lipoic acid group ($p < 0.05$, Tukey's post hoc test). c: Significantly different from the Cleome droserifolia group ($p < 0.05$, Tukey's post hoc test). d: Significantly different from the mercuric chloride group ($p < 0.05$, Tukey's post hoc test). e: Significantly different from the mercuric chloride and alpha-lipoic acid groups ($p < 0.05$, Tukey's post hoc test). f: Significantly different from both the mercuric chloride and Cleome droserifolia groups ($p < 0.05$, Tukey's post hoc test)

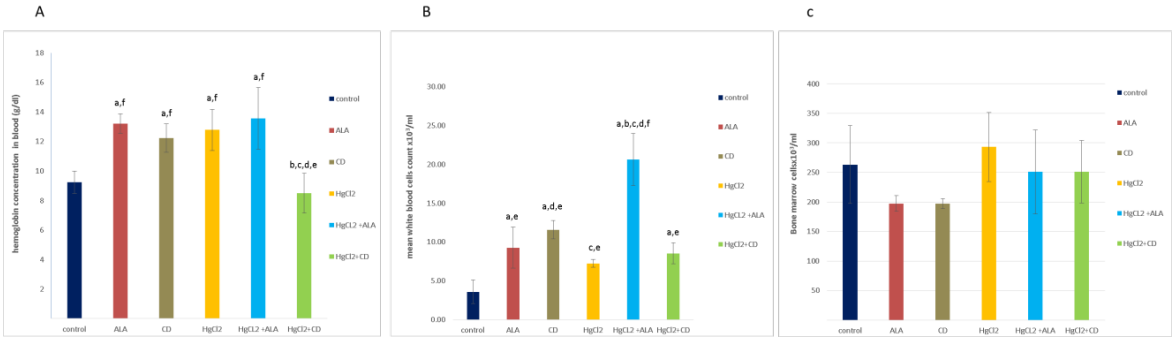

FIGURE 2. Effects of alpha-lipoic acid and Cleome droserfolia against mercuric chloride-induced effects on hemoglobin (A), total white blood cell count (B), and bone marrow cell count (C). HgCl2: mercuric chloride; ALA: alpha lipoic acid; CD: Cleome droserfolia; bw: body weight. Data are shown as mean ± SD for five rats per group. a: Statistically different from the control group ($p < 0.05$, Tukey's post hoc test). b: Statistically different from the alpha-lipoic acid group ($p < 0.05$, Tukey's post hoc test). c: Statistically different from the Cleome droserifolia group ($p < 0.05$, Tukey's post hoc test). d: Statistically different from the mercuric chloride group ($p < 0.05$, Tukey's post hoc test). e: Statistically different from the mercuric chloride and alpha-lipoic acid groups ($p < 0.05$, Tukey's post hoc test). f: Statistically different from the mercuric chloride and Cleome droserifolia groups ($p < 0.05$, Tukey's post hoc test).

Hematology

Mercuric chloride-induced a significant elevation in hemoglobin concentration by 38.31% compared to normal after 8 weeks of treatment (Figure 2A). However, *Cleome droserifolia* was more effective than lipoic acid in improving this effect. Total white blood cells significantly increased by 102.2% in the $HgCl_2$ group by 102.2% compared to the control group after 8 weeks of treatment (Figure 2B). Treatment with lipoic acid and CD exacerbated this effect. It could be noticed that bone marrow cell count is not affected in the different groups under investigation (Figure 2C).

Biochemistry

A significant ($p<0.05$) increase in MDA activity was observed in the $HgCl_2$-intoxicated group by 144.7% compared to normal controls after eight weeks of treatment (Figure 3A). ALA was found to reduce this effect better than *Cleome droserifolia*. Mercuric chloride induced a significant decrease in plasma catalase activity by 59.66% compared to normal rats after completing 8 weeks of treatment. On the other hand, administration of lipoic acid and *Cleome droserifolia* ameliorated this effect. However, lipoic acid is the most effective (Figure 3B).

A significant increase in mercury concentration was

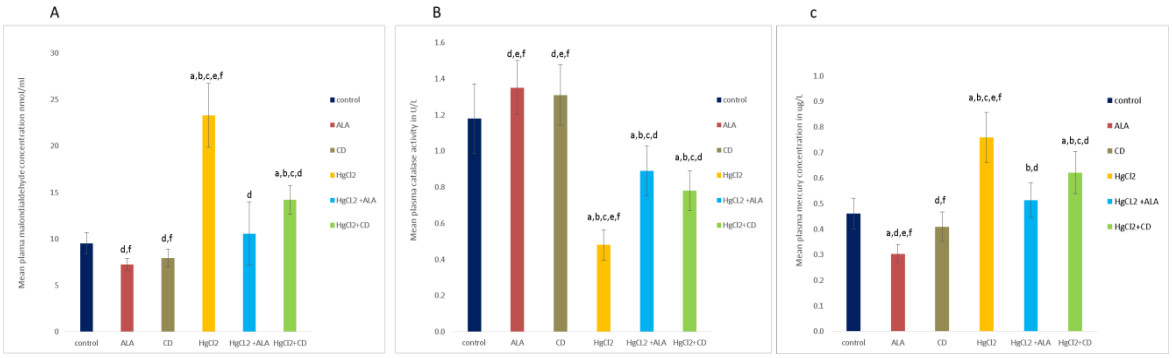

FIGURE 3. Effects of alpha-lipoic acid and Cleome droserfolia against mercuric chloride-induced alterations in plasma CAT activity (A), MDA concentration (B), and mercury concentration (C). HgCl2: mercuric chloride; ALA: alpha lipoic acid; CD: Cleome droserfolia; bw: body weight. Data are reported as mean ± SD for five rats in each group. a: Significantly different from the control group ($p < 0.05$, Tukey's post hoc test). b: Significantly different from the alpha-lipoic acid group ($p < 0.05$, Tukey's post hoc test). c: Significantly different from the Cleome droserifolia group ($p < 0.05$, Tukey's post hoc test). d: Significantly different from the mercuric chloride group ($p < 0.05$, Tukey's post hoc test). e: Significantly different from the mercuric chloride and alpha-lipoic acid groups ($p < 0.05$, Tukey's post hoc test). f: Significantly different from the mercuric chloride and Cleome droserifolia groups ($p < 0.05$, Tukey's post hoc test).

TABLE 1: Effects of mercuric chloride on liver and kidney function tests and the protective effects of alpha lipoic acid and *Cleome droserifolia* extract in male rats.

	AST (U/L)	ALT (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	117.4±15.82	38.4±5.22	40.8±5.06	0.73±0.089
ALA	80.410.92± a,d,e,f	23±3.16 a,d,e,f	13±1.58 a,c,d,e,f	0.56±0.076 d,e,f
CD	102±15.04 d,f	28±4.74 d,e,f	32.2±4.43 b,d,e,f	0.65±0.093 d,f
HgCl2	205.2±10.75 a,b,c,e,f	55.6±5.72a,b,c,e	81±9.027 a,b,c,e,f	1.2±0.117 b,c,e,f
ALA+ HgCl2	132.8±21.04 b,d	43.2±6.01 b,c,d	47±6.32 b,c,d,f	0.82±0.11 b,d
CD+ HgCl2	160±24.52 a,b,c,d	48.2±6.64 b,c	65.6±8.7 a,b,c,d,e	0.96±0.14 a,b,c,d

Data is presented as means ± SD for five rats per group. *a:* Significantly different from the control group ($p < 0.05$, Tukey's post hoc test). *b:* Significantly different from the alpha lipoic acid group ($p < 0.05$, Tukey's post hoc test). *c:* Significantly different from the Cleome droserifolia group ($p < 0.05$, Tukey's post hoc test). *d:* Significantly different from the mercuric chloride group ($p < 0.05$, Tukey's post hoc test). *e:* Significantly different from the mercuric chloride and alpha lipoic acid group ($p < 0.05$, Tukey's post hoc test). *f:* Significantly different from the mercuric chloride and Cleome droserifolia group ($p < 0.05$, Tukey's post hoc test). HgCl2 = mercuric chloride; ALA = alpha lipoic acid; CD = Cleome droserifolia; AST = aspartate aminotransferase; ALT = alanine aminotransferase

observed in the $HgCl_2$ group, amounting to 64.6% compared with normal controls after completing 8 weeks of treatment; however, using lipoic acid and *Cleome droserifolia* modulated this effect (Figure 3C). The data show that the plasma levels of Hg are lower in the ALA+Hg and CD+Hg groups compared to the Hg group. Plasma aspartate aminotransferase was increased significantly ($p<0.05$) under mercury toxicity for 8 weeks, with a magnitude of 74.78% compared with the control group. Both lipoic acid and *Cleome droserifolia* ameliorated this effect, but lipoic acid had the best effect (Table 1). Plasma ALT activity increased significantly by 44.79% compared to normal after completing 8 weeks of treatment. However, lipoic acid and *Cleome* droserifolia improved this effect, which was more evident with the lipoic acid supplement (Table 1). A significant elevation in creatinine concentration was observed in the group supplemented with mercuric chloride, which

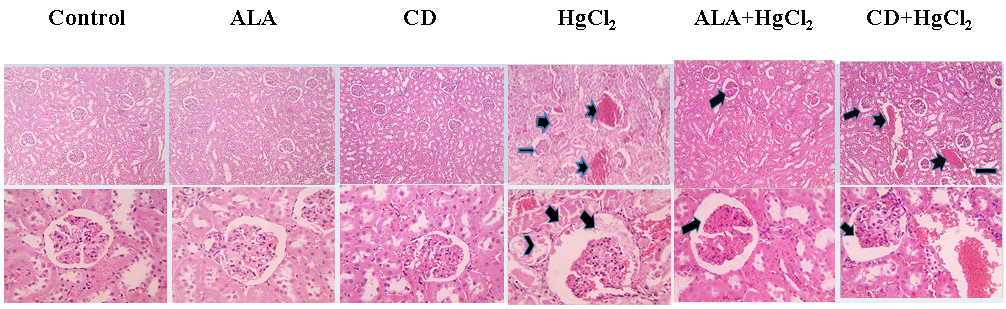

FIGURE 4. Effects of alpha-lipoic acid and *Cleome droserfolia* against mercuric chloride-induced alterations in kidney tissue. Microscopic pictures of Hematoxylin & Eosin stained kidney sections show normal glomeruli (G) and tubules (T) in the control group and the group receiving alpha-lipoic acid and *Cleome droserifolia*. Meanwhile, marked congestion was observed in kidney sections from the mercuric chloride group (thick arrows), swollen Bowman's capsule with eosinophilic proteinaceous material and shrunken glomerular tuft (thin black arrows), severe tubular hydropic degeneration (opened arrowhead) and coagulative necrosis (closed arrowheads). Renal sections from the treated group showed congestion (thick arrows), *Cleome droserifolia* + $HgCl_2$ showed a swollen Bowman's capsule, and a shrunken glomerular tuft (thin black arrows). Renal sections from the treated group $HgCI_2$+lipoic acid show a slightly swollen Bowman's capsule with a slightly shrunken and congested glomerular tuft (thin black arrows). Low magnification X: 100 bar 100 and high magnification X: 400 bar 50

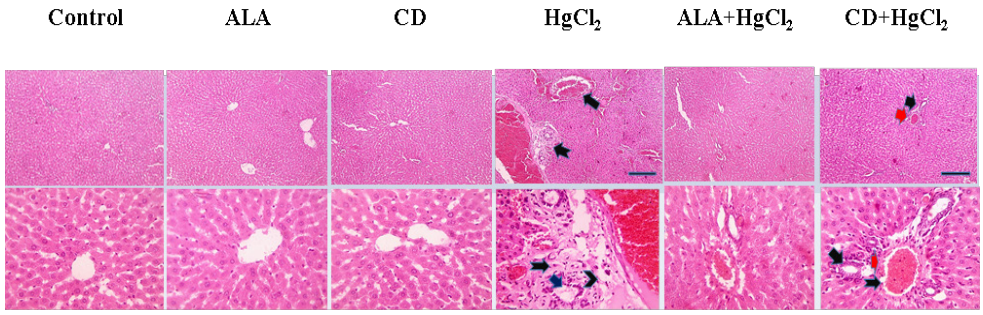

FIGURE 5. Effects of alpha lipoic acid and *Cleome droserfolia* against mercuric chloride-induced alterations in liver tissue. Microscopic pictures of Hematoxylin & Eosin stained liver sections show normal hepatocytes arranged in radiating plates around the central vein (CV) with normal portal areas and sinusoids in the control group and the groups receiving lipoic acid and *Cleome droserifolia*. Hepatic sections from group $HgCI_2$ showed marked congestion (red arrows), portal edema, and fibrosis (thick arrows) with bile duct proliferation (thin black arrows) and neutrophil infiltration (arrowheads). Hepatic sections from the $HgCI_2$+ Cleome droserifolia group showed markedly decreased congestion (thick arrows), portal fibrosis (thin arrows), and bile duct proliferation (arrowheads). Hepatic sections from the treated group $HgCI_2$+lipoic acid show mild portal vein congestion (red arrows). Low magnification X: 100 bar 100 and high magnification X: 400 bar 50.

amounted to 64.38% of normal controls after completing 8 weeks of treatment (Table 1). Both lipoic acid and *Cleome droserifolia* improved this effect, but lipoic acid had the best action. Mercuric chloride-induced significant increases in plasma urea concentration amounted to 98.5% higher than normal controls after completing 8 weeks of treatment (Table 1). Supplementation with lipoic acid and *Cleome droserifolia* improved this effect; lipoic acid was more effective.

Histopathological findings

Normal kidneys have a typical glomerular and tubular architecture and histological appearance. Mercuric chloride treatment resulted in significant tubular degeneration and necrosis eight weeks after exposure. Rats receiving lipoic acid and *Cleome droserifolia* supplements showed slight glomerular affection, less for lipoic acid than the plant extract (Figure 4).

The livers of normal rats showed normal hepatocyte architecture. Eight weeks after mercury ingestion, liver

tissue displayed congested blood vessels. It infiltrated white blood cells in the vicinity, portal edema, fibrosis, necrosis, and degeneration in hepatocytes after completing 8 weeks of treatment. Supplementation with alpha-lipoic acid and *Cleome droserifolia* improved these effects (Figure 5).

Discussion

Exposure to mercuric chloride resulted in a modest decrease in body weight (Figure 1A), which may be attributed to decreased food intake or nephrotoxicity (Mahboob et al., 2001; Haouem et al., 2015). Reducing body weight is a vital indicator of a rat's overall health disturbance, and the decline in organ weight is essential in evaluating organ toxicity (Uzunhisarcikli et al., 2016; Jaiswal et al., 2013). Mercury induces insignificant changes in body weight compared to the control group, consistent with the findings of Rao and Sharma (2001), who observed that body weight in adult male mice remained unaffected after 45 days of $HgCl_2$ treatment.

According to Uzunhisarcikli et al. (2016), increased liver weight may be caused by the body's adaptive mechanisms for mercury toxicity. Additionally, Caglayan et al. (2019) introduced 1.23 mg/kg of body weight of $HgCl_2$ daily for seven days to rats, and that didn't affect liver weights, which supports our results. It seems mercuric chloride's influence on liver weight is not due to certain behaviors.

Mercury exposure can damage the gastrointestinal (GI) tract (Rodriguez-Viso et al., 2023), leading to reduced food intake and malabsorption. Mercury can trigger oxidative stress and disrupt cellular metabolism (Dufault et al., 2009), causing increased protein catabolism (Lee et al., 2020). It can provoke a severe immune response, releasing cytokines such as tumor necrosis factor-alpha (TNF-α) and leading to neural cell death (Toyama et al., 2021). These toxic effects of Mercury may result in reduced nutrient absorption, muscle wasting, depletion of body fat reserves, and weight loss. Mercury induces fibrosis, apoptosis, and necrosis of hepatocytes (Wadaan, 2009; Vergilio et al., 2015, Debashis et al., 2019). As liver cells die and tissue shrinks, the overall liver weight decreases.

Mercury accumulates in the kidneys (Shalan 2022), leading to damage of renal tubules, which can cause tubular necrosis and glomerular damage (Figure 4), resulting in the shrinkage of the kidney due to cell death. Mercury enhances the disruption of the renal tubular system, reducing filtration capacity and impairing fluid and electrolyte balance (Talbott et al. 1937). Damaged tubular cells may die and slough off, further reducing kidney mass and weight. Chronic mercury exposure interferes with cellular repair mechanisms, preventing the regeneration of damaged kidney cells (Bridges and Zalups, 2017), further reducing kidney weight.

In the present investigation, it was observed that body weight and organ weight decrease proportionally. However, the relative organ weight may not change significantly or show only slight, statistically insignificant variations. Some tissues may be more sensitive to mercury toxicity than others. For instance, if body weight decreases due to muscle and fat loss while organ weights decrease slower, the relative organ weight may not show noticeable changes. Treatment of animals with lipoic acid and *Cleome droserifolia* elevated body weights significantly; this may be linked to the lipid nature of lipoic acid and the complex composition of Cleome *droserifolia*.

Elevated plasma levels of aspartate transaminase and alanine transaminase indicated impaired functioning of hepatocytes due to cellular necrosis and increased membrane permeability in mercury-exposed animals (Karuppanan et al., 2014). Xie et al. (2021) demonstrated that damage to hepatic cells can result in the release of ALT and AST, which correlate with the extent of hepatic cell damage. Eight weeks after mercury intake, liver tissue exhibited significantly congested blood vessels and infiltration of white blood cells around hepatocytes (Figure 5), providing insight into the behavior of white blood cells under mercury toxicity. Supplementation with both alpha-lipoic acid and *Cleome droserifolia* improved these effects.

From a histological perspective, $HgCl_2$ caused hepatic necrosis, hemorrhage, degeneration, and hyperemia (Caglayan et al., 2019). Mercuric chloride has been shown to enhance the expression of p53, caspase-3, and Bax, leading to significant morphological alterations. These changes include a reduction in ribosomes linked to the rough endoplasmic reticulum and an expansion of the Golgi apparatus (Achapelle et al., 1993). The toxicity of Mercury boosts the production of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide. These ROS cause oxidative stress, which damages hepatocyte cell membranes, induces lipid peroxidation,

and activates liver enzymes (Hussain et al., 1999; Jalili, 2019).

Based on Patel and Rao (2015) and Mumtaz et al. (2019), Mercury toxicity is linked to its ability to bind to thiol groups in various proteins and peptides, the decline in cellular antioxidants like SOD, glutathione (GSH), and CAT, and to promote the increase in MDA concentration. Plasma MDA content was significantly increased, and CAT was highly reduced after 56 days of mercuric chloride treatment (Figure 3A); however, supplementing with alpha lipoic acid and *Cleome droserifolia* at 10 mg/kg bw doses could potentially reduce these effects. Malondialdehyde is the final byproduct of lipid peroxidation, and its elevation indicates the production of free radicals under mercuric chloride toxicity. According to Su et al. (2008), MDA concentration reveals the proportion of damaged tissues and cells. Agarwal et al. (2010), Aslanturk et al. (2014), and Kalender et al. (2013) found elevated levels of MDA in various tissues and plasma under mercury intoxication. High MDA levels have been demonstrated to indicate damaged liver tissue (Uzunhisarcikli et al., 2016). Evidence showed that the cellular antioxidant defense mechanism (e.g., SOD and CAT) controls the potentially harmful effects of free radicals generated by mercuric chloride (Faix et al., 2003). The superoxide dismutase enzyme promotes the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen (Boujbiha et al., 2009). Catalase converts H2O2 into H2O and oxygen, safeguarding cells from oxidative damage (Renugadevi and Prabu, 2010). Oxidative stress is well known to be a factor in liver damage, regardless of the underlying etiology (Stankovi et al., 2014). ALA increases liver weight significantly (Figure 1A). Shay et al. (2009) concluded that ALA injection significantly decreased the weight of ZF livers, which is not in line with our findings Shay et al. (2009). They showed that ALA may have antioxidant properties and is hepatoprotective. Shaw et al. (2010) highlighted ALA's hepatoprotective antioxidant and anti-inflammatory properties in chronic hepatic fibrosis, cirrhosis, and acute liver injury.

Our results showed increased WBCs in rats that received mercury chloride (Figure 2B). The histopathological changes induced by Mercury could induce an elevation in WBC count (Kalender et al., 2010). Mercuric chloride causes abnormal leukocytosis, which may deteriorate the organ's tissues through an immune response (Mahour and Saxena, 2009). The results presented in Figure 2B indicate that administering alpha-lipoic acid and *Cleome droserifolia* extract alone significantly increases WBC counts, suggesting their potential immune-enhancing effects. The elevation of WBCs also points to their potential importance as antioxidant and anti-inflammatory mediators (Sztolsztener et al., 2022; Panicker et al., 2020). Alpha-lipoic acid and *Cleome droserifolia* may also impact cytokine production (Liu et al., El-Komy et al., 2017), which are signaling molecules that play a crucial role in the regulation and recruitment of immune cells, including WBCs.

Hemoglobin concentrations were significantly increased under the influence of mercuric chloride (Figure 2A). It was shown that mercuric chloride elevated RBCs and hemoglobin concentration by increasing the expression of erythropoietin, leading to enhanced activation of the Jak2/STAT5 signaling pathway in the bone marrow, which increased the number of erythrocyte progenitors in the bone marrow and did not affect the clearance of red blood cells He et al., (2021). Results from Ibegbu et al. (2014) and Shalan et al. (2023) showed that Mercury promoted a general decrease in erythrocytes and hemoglobin concentration. They attributed this to mercury-induced alterations in hemoglobin's oxygen-binding capacity, a decrease in band 3-mediated ion exchange, and an elevated risk of hemolytic and aplastic anemia due to Mercury competing with iron for binding to hemoglobin, which can hinder hemoglobin production. These findings suggest that the impact of mercuric chloride on hematologic indices is probably not linear.

The results of the present study (Table 1) showed significant elevations in liver and kidney functions (ALT, AST, uric acid, and creatinine). These elevations retained average values after treating the animals with ALA and CD. The marked increase in hepatic enzymes reflects poor liver function (Al-Attar, 2011). ALA increases the amount of cysteine in the liver while maintaining the activities of the glutathione metabolism enzymes (Konrad et al., 2001).

Plasma urea and creatinine serve as the primary measures of renal function. The elevation of these markers after exposure to Mercury demonstrates the negative impact of Mercury on kidney function. It is postulated that decreased renal excretion of these chemicals leads to glomerular injury and impaired renal function, resulting in elevated plasma urea and creatinine levels (Sala-

zar, 2014). Nabil (2014) found that exposure to Mercury decreases glomerular function. Naghibi et al. (2006) indicated that the body's antioxidant levels may decrease directly in response to elevated urea and creatinine levels. Higher urea levels can lead to oxidative stress by increasing the body's level of free radicalsNaghibi et al. (2006). Inorganic Mercury accumulation in the kidneys can result in acute renal failure (Tanaka-Kagawa et al., 1998). The uptake, buildup, and toxicity of inorganic Mercury in the kidney are associated with its affinity for endogenous thiol-containing compounds (Zalups et al., 2000). Mercury has been identified as a target for the thiol-containing enzyme aminolevulinate dehydratase (Emanuelli et al., 1996). A decrease in glutathione levels due to binding of mercuric ions to sulfhydryl groups can increase reactive oxygen species, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide (Stohs, 1995). By damaging biological substances, including DNA, proteins, and lipids, it alters enzyme function and leads to cell damage and death. Additionally, creatinine levels reflect the efficiency of the visceral layer of the Bowman's capsule in filtering substances and indicate hyperplasia in the proximal tubules (Rule et al., 2004).

This may be caused by increased intratubular pressure after protein casts clog the tubule, a direct mercury-related effect on the tubular basement membrane, or the alteration of the membrane, making it less elastic and more distensible. Mercuric chloride-enhanced tubular degeneration and necrosis were observed eight weeks after exposure. Rats subjected to mercuric chloride began to retain normal kidney architecture after receiving the lipoic acid supplement rather than *Cleome droserifolia* extract (Figure 4).

Zhang et al. (2016) showed that ALA prophylaxis increases glomerular filtration while lowering renal tubular injury indices, urine damage markers, kidney structural damage, and urinary damage scores. ALA can also reduce inflammation by focusing on NF-kB and reducing the release of inflammatory cytokines. Consequently, it has been demonstrated that ALA can enhance glomerular function and decrease renal inflammation by scavenging oxygen-free radicals (Gao et al., 2022). Studies have also shown that ALA therapy can lessen acute renal damage by reducing levels of tumor necrosis factor-alpha (TNF), interleukin-6 (IL-6), and interleukin-1 beta (IL-1), which in turn reduces endothelin-1 vasoconstriction, neutrophil diffusion, and inflammation in the kidneys. The accumulation of hydroxyl free radicals in the blood, liver, and kidney cortex was removed, and plasma urea and creatinine levels were decreased by lipoic acid. Treatment with α-lipoic acid and CD improved plasma urea, creatinine levels, malondialdehyde (MDA), and catalase activity (Table 1, Figure 3A, B). Data indicated that mercury exposure significantly elevated plasma mercury concentrations (Figure 3C). However, the ALA+Hg and CD+Hg groups exhibit lower plasma Hg levels than the Hg group. Since all treatments were administered orally, alpha-lipoic acid or *Cleome droserifolia* extract may decrease the oral absorption of Hg during co-administration. Due to this reduced absorption of Hg, lower toxicity is observed in the liver and kidney. The observed healing effect may be attributed to decreased mercury absorption rather than antioxidant effects at the tissue level.

It has been suggested that vitamin C, found in Cleome species, prevents the production of mutagenic electrophilic metabolites and stimulates 7-alpha-hydroxylation of lipids and cholesterol nuclei, increasing their degradation to bile acids that can be excreted from the body. Vitamin C could protect cells from oxidative damage through its antioxidant activity, decreasing cytotoxicity (Acharya et al., 2003).

Conclusion

The current study highlighted the modulatory effects of ALA and CD on mercury-induced hepatorenal toxicity, with the most pronounced modulation observed in rats supplemented with ALA rather than CD. Considering the results of this study, ALA and CD could be used as protective agents against mercuric chloride-associated hepatorenal toxicity.

Author contributions

Mohamed Gaber Shalan**:** conducted experimental design, all experimental procedures, measurements, and statistical analysis, and wrote and reviewed the manuscript.

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Conflicts of interest

The authors confirm that they have no competing interests.

Data availability

The datasets from this study are accessible from the corresponding author upon reasonable request.

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