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Beneficial effects of vitamin c supplementation against cadmium-induced hepatotoxicity in female *Swiss Albino* Mice

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ABSTRACT:
Cadmium, a toxic heavy metal and major environmental pollutant, is known for inducing oxidative damage in various organs, including the liver. This study was conducted to evaluate the protective effects of vitamin C against cadmium-induced oxidative liver damage in *albino* mice. Forty male mice were randomly divided into four groups of ten each. Group 1 served as the control and received a normal diet. Group 2 was treated with vitamin C (1 g/L ascorbic acid in drinking water). Group 3 received cadmium chloride (200 mg/L in drinking water), while Group 4 was exposed to cadmium chloride (200 mg/L in drinking water) along with vitamin C (1 g/L ascorbic acid in drinking water).

Exposure to cadmium in mice led to a significant reduction in body weight and an increase in liver weight compared to the control group. Cadmium treatment resulted in elevated levels of glucose, cholesterol, triglycerides, total lipids, bilirubin, and malondialdehyde (MDA), along with increased activities of glutamate-pyruvate transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), and alkaline phosphatase (ALP). Conversely, serum total protein levels, liver reduced glutathione (GSH), and catalase activities were markedly decreased.
In conclusion, the present study revealed that cadmium exposure exerts toxic effects on various biochemical and oxidative stress parameters. However, vitamin C supplementation significantly improved these parameters, highlighting its potential as a potent antioxidant. Vitamin C effectively mitigated the oxidative severity of cadmium-induced hepatotoxicity, likely through its ability to neutralize reactive oxygen species and enhance antioxidant defense mechanisms.
Keywords: Cadmium, vitamins C, liver, hepatotoxicity, GSH.

1. INTRODUCTION:

Heavy metals such as lead, cadmium are non-biodegradable elements and must therefore be extracted from polluted sites if they are to be eliminated (**Pilon-Smits, 2005 and Vangronsveld *et al.*, 2009**). The widespread exposure to heavy metals, driven by industrialization and human activities, poses serious health risks to both the environment and living organisms (**Bradl, 2005 and Briffa *et al.*, 2020**). Among these, cadmium (Cd) is

recognized as one of the most toxic heavy metals. This environmental and industrial pollutant is ubiquitously present in soil, water, air, and food (**Cinar, 2003 and Kaplan *et al*., 2011**). Environmental exposure to cadmium primarily occurs through contaminated drinking water, industrial emissions, vehicle exhaust, cigarette smoke, and agricultural products grown on polluted soils (**Pappas, 2011and Unsal *et al*., 2020**). It is extensively utilized in industrial processes, including the coating of polyvinyl chloride pipes, the manufacturing of plastics, glass, ceramics, rubber, paints, and fireworks (**Unsal *et al*., 2020**). Its distribution in the environment stems largely from atmospheric releases through waste incineration, steel production, and zinc manufacturing. Land contamination is primarily linked to improper waste disposal (**Ansari and Khan, 2015**). Biologically, cadmium targets major organs, particularly the kidneys and liver, where it induces metallothionein (MT) synthesis. The kidneys are considered the critical organ for Cd toxicity, often manifested as proximal tubular dysfunction (**Merali and Singhal, 1975**). Beyond nephrotoxicity, cadmium exposure leads to various health complications, including osteoporosis, cardiovascular disorders, testicular necrosis, renal failure, ocular damage, and neurological impairments (**Zhou *et al*., 2016 and Genchi *et al*., 2020**). Cadmium disrupts metabolic processes, affecting nucleic acids, carbohydrate metabolism, protein synthesis, and enzyme systems. It induces oxidative damage to lipid membranes, DNA, and proteins, resulting in cellular dysfunction and tissue injury. These effects alter the activity of antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase, and catalase, while promoting pollutant conjugation with glutathione-S-transferase (GSH). Additionally, cadmium exposure has been linked to the development of cancers, including those in the kidneys, lungs, testicles, and prostate (**Rousselet, 2007**). Antioxidant defense mechanisms protect against damage caused by reactive oxygen species (ROS) (**Irato and Santovito, 2021**). Ascorbic acid, also known as vitamin C, acts as an important enzyme cofactor. It is a low molecular weight, water-soluble compound that plays a key role in managing oxidative stress due to its strong antioxidant efficacy. Vitamin C can easily donate electrons, thus protecting essential biomolecules such as proteins, lipids, carbohydrates, and nucleic acids from oxidative damage caused by cellular metabolism. This vitamin also reduces inflammatory responses, which can affect numerous critical biological processes, thereby contributing to its immune-modulating effects (**Nimse and Pal, 2015 and Shati *et al*., 2021**). Many studies have investigated the role of oxidative stress and the ameliorative effects of antioxidant vitamins like vitamin C. These studies suggest that vitamin C, through its antioxidant mechanisms, may help mitigate stress-induced damage to cells and tissues. Vitamin C has been shown to protect against lipid peroxidation of

cytomembranes (**Retem *et al.*, 2021**). Additionally, it plays a significant role in neutralizing free radicals, protecting vital cells, and mitigating pesticide toxicity, particularly hepatic toxicity (**Shati *et al.*, 2021**). Furthermore, vitamin C is essential for collagen synthesis, neurotransmitter and specific hormone production, vitamin and amino acid metabolism, systemic toxin elimination, and the proper functioning of the immune system (**Karmmon *et al.*, 2011**). Research by **KarabulutBulan *et al.*, (2008)** demonstrated that administering vitamin C to rats (250 mg/kg per day) one hour before cadmium exposure for eight consecutive days significantly attenuated cadmium-induced kidney oxidative injury. Similarly, (**Shaban El-Neweshy and Said El-Sayed, 2011**) reported that vitamin C pretreatment protected against long-term exposure to lead-induced histopathological changes in the kidneys of rats.

The present study aims to evaluate the potential protective role of vitamin C in mitigating hepatotoxicity induced by cadmium chloride. To validate this objective, we propose two primary approaches:

-A hematological analysis involving the measurement of key biochemical parameters such as glucose and cholesterol…..

- An investigation of oxidative stress by assessing glutathione (GSH) levels.

2. MATERIALS AND METHODS:

1- Chemicals:

Vitamin C, cadmium chloride ($CdCl_2$) were purchased from sigma Chemical Co. (St Louis, France), and all other chemicals used in the experiment were of analytical grade.

Female *albino* mice with a body weight of 192to 209 g were obtained from Pasteur Institute (Algiers, Algeria). Animals were acclimated for two weeks under the same laboratory conditions of photoperiod (12 h light/12 h dark) with a relative humidity of 40% and room temperature of 22 ± 2°C. Food and water were available ad-libitum.

Experimental design Animals were randomly divided into four groups (ten each). One served as normal control group (T). The Second group (Vit C) was treated with vitamin C (1 g/L ascorbic acid in water). The third group (Cd) was given cadmium at quantity 200 mg/L in their drinking water as cadmium. The fourth group received was given cadmium at quantity 200 mg/L in their drinking water as cadmium and was treated with vitamin C (1 g/L ascorbic acid in water) (Cd-Vit C).The doses of Cd and Vit C used in the present experiment were selected on the basis of previous studies of (**Chowdhury *et al.*, 1987 and Yildirim and**

Büyükbingöl , 2003) respectively. The experimental procedures adhered to the National Institute of Health Guidelines for animal Care and were approved by the Ethics Committee of our Institution. Treatments were administered to the rats for a period of four weeks, during which body weight and food intake were regularly recorded. At the end of the experiment, the animals were sacrificed by decapitation without anesthesia to minimize stress. Blood samples were collected in appropriate tubes and centrifuged at 3000 rpm for 10 minutes to separate the serum. The obtained serum was utilized to analyze various biochemical parameters, including glucose, cholesterol, triglycerides, total lipids, bilirubin, glutamate-pyruvate transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), alkaline phosphatase (ALP) activities, and total proteins.

2- Measurement of hepatic oxidative stress parameters:

2-1 Tissue preparation and Oxidative Stress Parameters:

About 1 g of liver was homogenized in 2 ml of buffer solution of phosphate-buffered saline 1:2 (w/v; 1 g tissue with 2 ml TBS, pH 7.4). Then, the homogenates were centrifuged at ×10,000 g for 15 min at 4°C and the resultant supernatant was used for the determination of MDA, GSH , CAT and liver proteins.

3- Analytical methods:

3-1 Determination of biochemical parameters:

Glucose, GPT, GOT, ALP, total proteins, and direct bilirubin were assessed using Spinreact Laboratory Spain diagnostic kits and spectrophotometer (Jenway 6505, Jenway LTD and Essex, UK). The references of kits were as follows: Glucose-41011, GOT-1001161, GPT-1001171, ALP-1001131, total proteins-1001291, and direct bilirubin-1001041.

3-2 Estimation of Lipid Peroxidation (MDA):

The level of lipid peroxidation in liver homogenates was measured as malondialdehyde(MDA), the end product of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) to form a red-colored complex, with peak absorbance at 532 nm, following the method described by **Buege and Aust (1984)**.

3-3 Estimation of Reduced Glutathione (GSH):

liver GSH content was determined using a colorimetric technique described by **Ellman, (1959)** and modified by **Jollow *et al.*, (1974)**. This method relies on the formation of a yellow color when DTNB reacts with sulfhydryl groups. Briefly, 0.8 ml of liver supernatant was

mixed with 0.3 ml of 0.25% sulfosalicylic acid, followed by centrifugation at 2500 x g for 15 minutes. The resulting supernatant (0.5 ml) was combined with 0.025 ml of 0.01 M DTNB and 1 ml of phosphate buffer (0.1 M, pH 7.4). The absorbance was recorded at 412 nm, and total GSH content was expressed as nmol GSH/mg protein.

3-4 Assay of Catalase (CAT):

Catalase activity was determined using the method of **Aebi *et al*., (1984)**. The reaction mixture (1 ml) consisted of 0.78 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml liver supernatant, and 0.02 ml H_2O_2 (0.5 M). The reaction was initiated by adding H2O2, and the decomposition rate was monitored as a decrease in absorbance at 240 nm for 1 minute. Enzyme activity was calculated using an extinction coefficient of 0.043 $mM^{-1}cm^{-1}$.

3-5 Protein Determination:

The protein content in liver tissue was quantified using the **Bradford method, (1976)** with bovine serum albumin as the standard.

4- Statisical Analysis:

Data are presented as means ± SEM. Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Student's t-test, with a significance level set at $p < 0.05$.

3. RESULT:

3-1- Effect of treatment on body, liver weights and food intake:

The findings illustrated in table 1 showed that body weight and food intake of animals exposed to Cd was significantly decreased by ($p < 0.001$) as compared to controls. In addition, a significant increase of absolute and relative liver weights in Cd treated group was noticed ($p < 0.001$) **(Table 1)**. However, the administration of Vit C restored most the previous mentioned parameters.

Table 1: Initial body weight, final body weight, food intake, absolute and relative liver weights of control rats, treated with Vitamine C (Vit C), Cadmuim (Cd), and Cadmuim with Vitamine C (Cd-Vit c) after four weeks of treatment (*Values are given as mean± SEM, n= 10, ***p<0.001*).

Parameters	Experimental group			
	Control (n=10)	**Vit C (n=10)**	**Cd (n=10)**	**VitC-Cd (n=10)**
Initial body weight (g)	192±9.35	193±11.19	209±14.34	208±9.41
Final body weight (g)	248±11.51	219±47.78	170±19.155***	222±9.525***
Liver Absolute weight (g)	6.77±1.27	6.86±1.40	1.33±1.07***	7.84±1.04
Liver Relative weight g/100g.bwt	16.88±3.53	15.74±3.93	24.31±2.09***	17.46±2.50
Food intake(g/day/rat)	22±2.59	22±2.63	11±2.25***	15±1.95***

3-2- Effect of treatment on serum biochemical parameters:

Treatment with Cd caused significant increase of serum glucose, Total lipids, GOT, GPT and ALP ($p < 0.001$); highly significant ($p<0.01$) for Cholesterol and significant ($p<0.05$) for Triglyceride, Direct bilirubin. Meanwhile, the concentration of serum total protein was diminished ($p<0.05$) compared to the control group **(Table 2)**.

However, the administration of Vitamin C restored most the previous mentioned parameters.

Table2: Changes of biochemical parameters of control and treated mice with Cd, Vit C, Cd - Vit C after 4 weeks of treatment (*Values are given as mean± SEM, n= 10, *P < 0.05, **P < 0.01, ***p<0.001*).

Parameters	Experimental group			
	Control (n=10)	**Vit C (n=10)**	**Cd (n=10)**	**VitC-Cd (n=10)**
Triglyceride (mg/dl)	71,03±26,23	72,19±33,48	90,87±4,99*	76,74±14,62
Cholesterol (mg/dl)	68.66±14.17	66.66±4.38	93.83±8.13**	74.66±9.56
Glucose (g/l)	1.01±0.16	1.04±0.16	1.25±1.0.09***	1.16±0.13
Total lipids (mg/dl)	336.16±12.65	331.83±18.08	411.83±38.46***	354.83±54.31
Total protein (mg/dl)	8.33±1.08	8.41±0.72	6.85±1.04*	7.36±0.69*
Direct bilirubin (mg/dl)	0.25±0.20	0.26±0.15	0.44±0.12*	0.34±0.16
GPT	44.33±13.85	46.08±9.00	74.03±10.05***	52.81±2.79
ALP	115±3.84	116±4.63	136.33±7.68***	122±5.51*
GOT	75.05±4.26	73.82±3.53	118.32±8.76***	92.95±16.55*

3-3- Effect of treatment on hepatic oxidative stress parameters:

As shown in **Figure1**, the exposure to Cd produced a significant adverse effect on the liver redox status, which is indicated by an increase ($p<0.05$) of MDA level and a decrease of GSH, CAT ($p<0.001$) content. However, supplementation with Vit C in association with Cd ameliorated most these stress oxidative biomarkers.

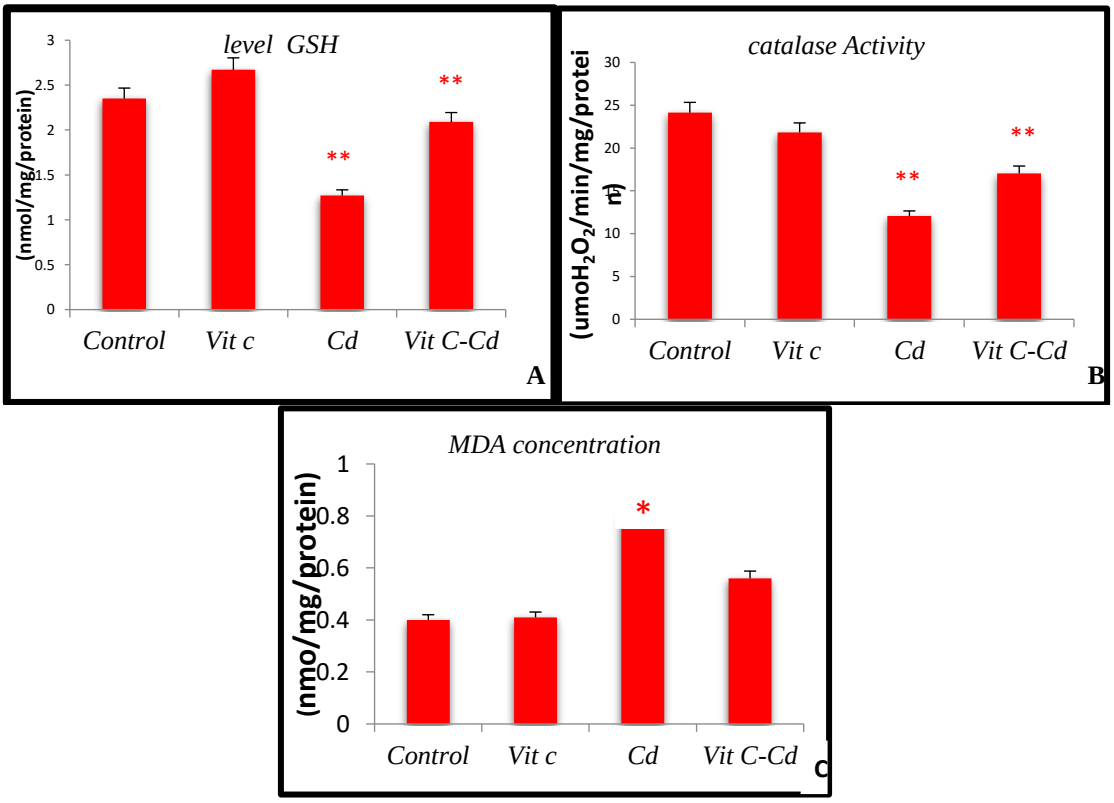

Figure 1: (A, B, C) Liver glutathione concentrations , catalase activity and MDA of control rats and treated mice with Cd, Vit C and Cd -Vit C after 4 weeks of treatment (*Values are given as mean± SEM, n= 10, *P < 0.05, **P < 0.01*).

4. DISCUSSION:

The liver is a primary target organ for cadmium (Cd) toxicity (**Layachi and Kechrid, 2012 and Gueroui and Kechrid, 2016**). Epidemiological studies on chronic liver diseases, including liver fibrosis, nonalcoholic fatty liver disease (NAFLD), and cirrhosis, have consistently shown elevated cadmium levels in affected individuals. These findings indicate a strong positive correlation between cadmium exposures and the onset or progression of chronic liver diseases (**Kolachi *et al.*, 2012 and Sun *et al.*, 2024a**). The toxic effects of metals on organisms can be influenced by exposure to trace elements (e.g., zinc, magnesium, selenium) or vitamins (e.g., vitamin C, vitamin E) (**Lakbar *et al.*, 2016 and Kouadria *et al.*, 2020**). Vitamin C, a potent antioxidant, is renowned for its ability to mitigate oxidative liver damage caused by various xenobiotics. This study aimed to assess the protective effects of

vitamin C against cadmium (Cd)-induced oxidative liver injury in albino mice. The findings revealed a significant reduction in body weight in the group exposed to Cd, aligning with previous studies on Cd toxicity (**Poli *et al*., 2022**). This weight loss is likely due to increased lipid and protein degradation (**Wang *et al*., 2016**) and reduced appetite (**Derbal and Kechrid, 2019**). Cd toxicity also causes pain, distress, and inflammation, reducing movement and appetite, which can result in anorexia, food avoidance, or diminished food palatability. Additionally, Cd-induced oxidative stress disrupts the antioxidant defense system, contributing to severe metabolic disorders and weight loss. Previous studies have similarly reported that inflammation resulting from Cd toxicity or treatment can cause weight loss ranging from 1–20% (**Renuka *et al*., 2021**). Many studies have reported that vitamin C, zinc, and N-acetylcysteine (NAC) are highly effective in mitigating Cd-induced toxicity. These compounds play a crucial role in restoring metabolic balance, enhancing food intake, and improving body weight, organ weight, and overall physiological health to the maximum possible extent (**Poli *et al*., 2022**).

The results revealed elevated blood glucose levels in animals exposed to cadmium (Cd), underscoring its significant metabolic impact. Studies in animal models of Cd exposure have shown that Cd disrupts glucose transport in both insulin-independent and insulin-dependent tissues by directly affecting the pancreas. Cd alters pancreatic morphology, impairs β-cell function, and induces oxidative damage, a critical factor in the development of diabetes (**Jacquet *et al*., 2016**). This hyperglycemia is primarily attributed to Cd's toxic effects, including the disruption of pituitary gland secretory activity, inhibition of insulin production by targeting the islets of Langerhans (**Massanyi *et al*., 1995 and Ambali *et al*., 2011**), and impairment of glucagon secretion, which increases glycogen breakdown and gluconeogenesis from proteins (**Massanyi *et al*., 1995**). However, Cd-induced hyperglycemia is ameliorated in animals treated with vitamin C, as reported by **Layachi and Kechrid, (2012)**, **Dahdouh *et al*., (2013),** and **Dawood *et al*., (2024).** Vitamin C, along with vitamin E, may enhance glucose absorption by modifying insulin receptor activity and increasing membrane permeability in muscle and adipose tissues.

The present study demonstrated a significant increase in serum total bilirubin levels in cadmium-intoxicated animals, consistent with findings by **Renugadevi and Prabu (2010)**. Elevated bilirubin levels serve as a clear indicator of hepatic dysfunction and are accompanied by a rise in hepatic marker enzymes, signaling liver damage and impaired

function (**Milton *et al*., 2008 and Renugadevi and Prabu, 2010**). Cadmium exposure also led to a decrease in serum total protein, likely resulting from disruptions in protein synthesis and/or metabolism. Hypoproteinemia in cadmium-treated animals may stem from dietary insufficiency, increased protein excretion rates, or hepatic inflammation, which interferes with protein biosynthesis (**Chawla, 2014 and Al-Baqami and Hamza, 2021**). These findings align with previous research (**Layachi and Kechrid, 2012 and Poli *et al*., 2022**).

Additionally, a significant increase in lipid profile parameters, including cholesterol, triglycerides, and total lipids, was observed in cadmium-exposed rats, corroborating the results of **Alese *et al*., (2020)**. Chronic cadmium administration induced hypercholesterolemia, which may result from hypothyroidism, hyperlipoproteinemia, or liver toxicity (**Kim *et al*., 2007 and Sun *et al*., 2024a**). Elevated cholesterol levels have critical health implications due to their role in atherosclerosis, a leading cause of mortality in developed nations (**Daniels *et al*., 2009 and Udi *et al*., 2022**). However, cadmium-contaminated rats treated with vitamins C and E exhibited regulated serum lipid parameters, highlighting the protective effects of these antioxidants in mitigating lipid dysregulation and liver toxicity. This appears to decrease hepatic lipogenic enzyme activity, improve insulin action, or increase lipoprotein lipase activity (**Paolisso *et al*., 1989**).

The present study revealed a significant increase in serum GOT, GPT, and alkaline phosphatase activities, indicative of hepatic damage. This elevation can be attributed to the release and leakage of these enzymes from liver cells into the bloodstream due to cadmium-induced hepatotoxicity (**Pari and Murugavel, 2005**). Antioxidants, such as vitamins C and E, have been shown to stabilize hepatic cell membranes, protecting hepatocytes from cadmium's toxic effects and reducing enzyme leakage into the plasma (**Layachi and Kechrid, 2012**). Additionally, the administration of vitamins C and E to cadmium-exposed animals slightly elevated protein levels, bringing them closer to normal. The observed reduction in antioxidant enzyme activities aligns with findings from previous studies, which highlight similar disruptions caused by oxidative stress in cadmium toxicity. (**Das and Al-Naemi *et al*., 2019; Renuka *et al*., 2021; Poli *et al*., 2022; Laib *et al*., 2024 and Zou *et al*., 2024**). The results of the study revealed significant increases in MDA levels and decreases in GSH and CAT activities in rats treated with cadmium, consistent with findings from **Poli *et al*., (2022) and Laib *et al*., (2024)**. Several mechanisms have been proposed to explain the depletion of GSH in heavy metal toxicity. First, the sulfhydryl group in glutathione strongly

binds to metals, forming stable mercaptide complexes that can be excreted through bile. Additionally, GSH may be oxidized due to its interaction with free radicals, particularly those induced by metals such as nickel, leading to its consumption during detoxification (**Manna *et al*., 2008**). The increased hepatic lipid peroxidation (LPO), as evidenced by elevated MDA levels in cadmium-treated animals, suggests significant liver and kidney damage due to cadmium toxicity. The tissue's higher affinity for cadmium and the presence of cytochrome P450 further support the notion of cadmium's detrimental effects. These findings align with earlier studies, which reported that cadmium disrupts both the structural and functional integrity of the liver and kidneys. MDA, a marker of lipid peroxidation, has been widely used in previous research to monitor oxidative stress and evaluate damage to lipid components (**Poli *et al*., 2022**).

In the present study, lipid peroxidation (LPO) was chosen for precise measurement of oxidative damage, and the results showed an increase in LPO levels in the liver and kidney tissues of cadmium-exposed animals, indicating intensified lipid peroxidation and heightened oxidative stress. Organisms typically have an endogenous antioxidant defense system, including enzymatic antioxidants such as SOD, CAT, and GPx, which play crucial roles in detoxifying harmful oxygen metabolites. The failure of this primary antioxidant system to neutralize free radicals generated during oxidative stress may indicate the liver mitochondria and microsomes' inability to eliminate hydrogen peroxide (H2O2) produced after cadmium exposure (**Poli *et al*., 2022**). This failure may also stem from enzyme inactivation caused by the excessive production of reactive oxygen species (ROS) within the mitochondria and microsomes, leading to further oxidative damage (**Giuseppe *et al*., 2009**).

The study also revealed that cadmium exposure resulted in significantly higher MDA levels and lower GSH and CAT levels. However, when treated with vitamin C, these parameters were normalized, with a significant reduction in MDA and an increase in GSH and CAT levels. This is consistent with previous findings suggesting that vitamins C and E can reduce lipid peroxidation and increase antioxidant enzyme activities (**Layachi and Kechrid, 2012**). The normalization of GSH, GSH-Px, and catalase activities following vitamin C treatment reflects the ability of these vitamins to mitigate oxidative stress and reduce ROS accumulation. This was also observed in rats treated with vitamin C, zinc, and NAC, individually or in combination, where an increase in CAT activities was observed despite cadmium exposure. Furthermore, the interaction between cadmium and essential trace

elements, such as zinc, may contribute to reduced antioxidant enzyme functionality. Cadmium, by replacing zinc in Cu/Zn-SOD, forms inactive enzyme variants (Cu/Cd-SOD), which compromise enzymatic activity (**Bauer *et al*., 1980**).

5. CONCLUSION:

Vitamin C plays a significant protective role against cadmium (Cd) toxicity, primarily due to its antioxidant properties.Vitamin C reduces oxidative stress by scavenging reactive oxygen species (ROS) and restoring reduced glutathione (GSH) levels. It also protects liver function by decreasing lipid peroxidation, enhancing the activity of antioxidant enzymes such as catalase (CAT), and normalizing biochemical markers like ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), ALP (Alkaline Phosphatase), and bilirubin.Moreover, Vitamin C helps rebalance lipid and protein metabolism, reduces cadmium accumulation in vital organs, and alleviates the immunosuppressive and hepatotoxic effects of this metal. However, further studies are needed to optimize its doses, explore its interactions with other antioxidants, and maximize its therapeutic benefits

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