American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

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Systemic Influence of EDTA Used as a Preservative on Neurological Hematological and Oxidative Stress Responses in Male Albino Mice

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To Cite This article: *Javeria Zahra, Aiman Mazhar1, Farhat Siddique, Sumbal Ayyaz, Haq Nawaz, Rimsha Batool and Tanzila Rehman*, Systemic Influence of EDTA Used as a Preservative on Neurological Hematological and Oxidative Stress Responses in Male Albino Mice. Am J Biomed Sci & Res. 2026 32(5) AJBSR.MS.ID.004207,* **DOI:** 10.34297/AJBSR.2026.32.004207

Received: August 09, 2026 **Published:** November 10, 2026

Abstract

This study investigates the effects of synthetic EDTA on neurobehavioral, hematological, and oxidative stress markers in male albino mice. Mice were divided into control, Low-Dose (LSD), and High-Dose (HSD) groups and treated via oral gavage for nine days. Neurobehavioral assessment using the light-dark box test revealed that EDTA treatment significantly improved exploratory behavior, as mice spent more time in the light. Hematological analysis showed significant increases in WBCs, RBCs, Hematocrit (HCT), and Platelet (PLT) counts, suggesting potential impacts on immune function, red blood cell homeostasis, and coagulation. However, hemoglobin, lymphocyte counts, and lymphocyte percentage remained statistically unchanged. Oxidative stress Markers Indicated Decreased Lipid Peroxidation (MDA), Reduced Antioxidant Enzyme Activities (SOD, CAT, GPx), and depletion of Reduced Glutathione (GSH), reflecting compromised cellular defense mechanisms. Biochemical analysis showed no significant alterations, except for a decrease in LDL levels. Energy-Dispersive X-ray (EDX) analysis confirmed the absence of sodium accumulation in liver, kidney, and lung tissues, suggesting that EDTA did not accumulate in these organs. While EDTA is widely used as a preservative and chelating agent, these findings highlight its potential oxidative effects, emphasizing the need for further research to evaluate long-term safety.

Keywords: Food preservative, EDTA, Oxidative stress, Lipid peroxidation, Antioxidant enzymes, Neurobehavioral analysis, Biochemical assessment

Introduction

Various food groups are classified based on their nutritional composition in biological contexts [11]. These groups typically include dairy products, such as milk derivatives, fruits like bananas and oranges, and vegetables such as spinach, carrots, and onions [28]. Additionally, beans, legumes, and grains are integral components of these classifications [7]. Water is also recognized as an essential element in dietary guidelines, fulfilling multiple physiological and culinary functions [16]. Natural preservatives, which do not exhibit harmful effects, encompass substances such as sugar, salts, rosemary extract, and vinegar [22]. Several non-

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chemical preservation techniques, including boiling, pickling, dehydration, pasteurization, freezing, and smoking, are widely employed to extend the shelf life of food products [26,33]. However, dehydration is a standard method used for preserving coffee powder and soups, allowing for prolonged storage [27]. The elimination of air, microbial contaminants, and moisture further enhances microbial control in food preservation [6]. Artificial preservatives primarily function to inhibit microbial activity and proliferation, thereby prolonging the stability and shelf life of food products [24]. This category includes antimicrobial agents and antioxidants. Antimicrobials prevent microbial action, whereas antioxidants protect food components from oxidation [4]. Common antimicrobial preservatives include benzoates, nitrates, calcium propionate, sodium benzoate, and Ethylenediaminetetraacetic Acid (EDTA) [29]. Antioxidants such as Butylated Hydroxytoluene (BHT), Butylated Hydroxyanisole (BHA), formaldehyde, and ethanol are widely used to prevent oxidative deterioration in food products [12].

EDTA is extensively utilized as a preservative, particularly in ophthalmic solutions. Initially, it was suspected to contribute to acute allergic conjunctivitis and perioral dermatitis [19,20]. EDTA and its edetate salts serve as chelating agents in pharmaceutical formulations, cosmetics, and food products. These compounds form stable, water-soluble complexes with alkaline earth and heavy metal ions, with the stability of the metal-edetate complex depending on the nature of the bound metal ion and the pH of the solution [13]. The calcium-EDTA complex exhibits relatively weak binding and preferentially chelates heavy metals such as iron, cadmium, copper, and lead, facilitating their excretion through urine [13,34]. In cases of lead poisoning, calcium disodium EDTA is employed as a therapeutic agent [34]. Recent studies have reported the addition of calcium EDTA to nebulizer solutions for asthmatic patients undergoing COVID-19 treatment using simple mechanical ventilators [32]. This application was based on the hypothesis that EDTA, a component of several cardiac drugs, could contribute to viral mitigation through calcium release in conjunction with β-2 agonists [5]. EDTA has been recognized for its multifunctional role as an anticoagulant, chelating agent, and excipient, suggesting potential synergistic effects in its use as an artificial preservative and beyond [18]. Furthermore, the U.S. Food and Drug Administration (FDA) regulatory guidelines recommend the use of only a single type of synthetic preservative in each food preservation process [3]. In this study, we investigated the toxicity of EDTA in a murine model to evaluate its potential synergistic effects, thereby validating our research hypothesis.

Materials and Methods

Experimental Animals and Ethical Approval

Adult male BALB/c albino mice (4–5 weeks old, 25–30 g) were obtained from the Animal facility of Bahauddin Zakariya University (BZU), Multan, Pakistan. Animals were housed under controlled environmental conditions: 22±1°C temperature, 55–65% humidity, and a 14:10-hour light–dark cycle, with adlibitum access to standard rodent chow and water. Mice were acclimatized for four days in individual sanitized polypropylene cages with 5 cm-thick cotton bedding to minimize stress and aggression. Adult male BALB/c albino mice were maintained under controlled conditions as described previously [31]. All procedures were approved by the Institutional Animal Ethical Committee the Women University Multan. All experimental procedures were conducted in compliance with institutional ethical guidelines and were approved by the Institutional Animal Ethical Committee, The Women University Multan (Approval No. WUM/IAEC/2023/017).

Experimental Design and Treatment

Following acclimatization, mice were randomly assigned to three groups (n=6 per group):

a. Control Group: Received 0.9% saline solution

b. Low-Dose EDTA Group (LSD1–LSD6): Received 0.05% EDTA solution

c. High-Dose EDTA Group (HSD1–HSD6): Received 0.15% EDTA solution

All treatments were administered orally via gavage once daily for the entire experimental period (as specified). Dosage volumes were adjusted according to individual body weights. Throughout the study, animals were monitored for general health, body weight changes, and any behavioral abnormalities. Randomized treatment groups and oral gavage administration followed standard protocols [30].

Body Weight Monitoring

Body weight was recorded at baseline and every day throughout the experimental period (for nine days) to monitor changes in response to EDTA administration [35]. Weight data were used to evaluate potential treatment-related metabolic or physiological alterations.

Light–Dark Box Test

The light–dark box test was performed to evaluate anxiety-like behavior [25]. To assess anxiety-like behavior, each mouse was placed in the illuminated compartment of a standard two-chamber light–dark box apparatus (dimensions: 44 × 21 × 21 cm), and behavior was recorded for 5 minutes. The duration spent in the dark compartment was used as an index of anxiety, with longer times indicative of heightened anxiety levels. The apparatus was cleaned with 70% ethanol between trials to eliminate odor cues.

Hematological and Serological Analysis

Blood collection and hematological analysis followed standard protocols [17]. ELISA procedures were performed according to manufacturer instructions MyBioSource (2023). The blood samples were collected via retro-orbital puncture under mild isoflurane anesthesia. Samples were divided into two portions:

a. One portion was transferred to EDTA-coated tubes for hematological analysis using an automated hematology

analyzer (Sysmex XP-300, Japan) to determine parameters such as Hemoglobin (Hb), Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC), and platelet count.

b. The second portion was centrifuged at 3000 rpm for 15 minutes at 4°C to isolate serum. The collected serum was stored at −20°C until analysis. Levels of inflammatory cytokines (IL-6, TNF-α) and biochemical markers (ALT, AST, creatinine, urea) were quantified using commercial ELISA kits (MyBioSource, USA) according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (BioTek ELx800, USA).

Lipid Peroxidation (malondialdehyde, MDA)

At the end of the treatment period, brain and blood samples were collected, homogenized in ice-cold phosphate-buffered saline (PBS, pH 7.4), and centrifuged at 10,000 rpm for 10 minutes at 4°C. The resulting supernatants were used for the biochemical estimation of oxidative stress markers. Lipid peroxidation was determined by quantifying Malondialdehyde (MDA) levels using the Thiobarbituric Acid Reactive Substances (TBARS) assay as described by (Saeed, et al., 2024) [21]. Briefly, the reaction mixture containing tissue homogenate, 1% phosphoric acid, and 0.6% Thiobarbituric Acid (TBA) was incubated at 95°C for 60 minutes. After cooling, absorbance was measured at 532 nm using a UV–Visible spectrophotometer. MDA concentration was calculated from a standard curve prepared with 1,1,3,3-tetramethoxypropane and expressed as nanomoles of MDA per milligram of protein. Protein content in the supernatants was determined separately using the Bicinchoninic Acid (BCA) method for normalization.

Antioxidant Enzyme Activities

Antioxidant enzyme activities were measured to evaluate the redox balance in tissue samples. Superoxide Dismutase (SOD) activity was assessed based on its ability to inhibit the photochemical reduction of Nitroblue Tetrazolium (NBT), and absorbance was measured at 560 nm. Catalase (CAT) activity was evaluated by measuring the rate of decomposition of hydrogen peroxide (H_2O_2) at 240 nm. Glutathione Peroxidase (GPx) activity was determined using a coupled assay involving reduced Glutathione (GSH) and NADPH, with absorbance read at 340 nm. Intracellular GSH levels were quantified using Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid, DTNB) at 412 nm. SOD, CAT, GPx, and GSH activities were assayed following protocols outlined in previous studies [1,23].

Statistical Analysis

All statistical analyses were performed using SPSS software. Results were presented as mean ± Standard Deviation (SD). Differences among treatment groups were Assessed Using One-Way Analysis of Variance (ANOVA), followed by post-hoc tests where applicable to identify specific intergroup differences. A p-value of less than 0.05 was considered statistically significant, while values exceeding this threshold were regarded as non-significant.

Results

Body Weight

A significant reduction in body weight was observed in EDTA-treated mice compared to the control group (Figure 1). The control group maintained stable weights, whereas both Low-Dose (LSD) and High-Dose (HSD) groups showed a consistent decrease over the treatment period. This decline was statistically significant (p = 0.05), suggesting a dose-dependent impact of EDTA on body mass regulation or metabolism (Figure 1).

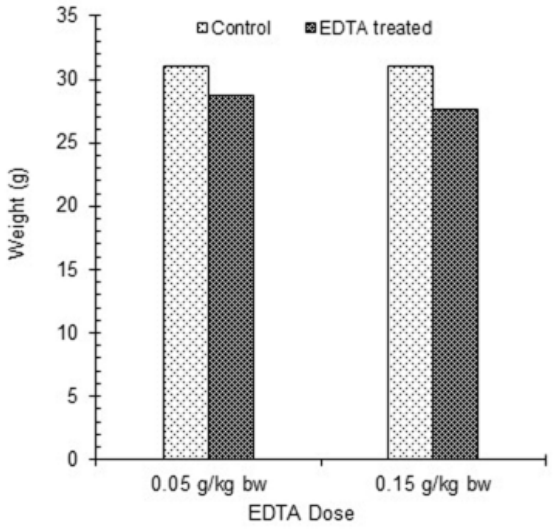


Figure 1: Weight analysis of mice.

Neurobehavioral Assessment (Light and Dark Box Test)

Behavioral analysis (Table 1) revealed significant changes in anxiety-like responses. Time spent in the Dark Compartment (TD) decreased substantially with EDTA treatment, from 261.5 ± 32.9 seconds in controls to 161.00 ± 19.97 seconds in the LSD group and 83.66 ± 11.59 seconds in the HSD group (p = 0.000). Conversely, time spent in the Light Compartment (TL) increased from 38.50 ± 32.9 seconds in the control to 139.0 ± 19.97 seconds in LSD and 216.33 ± 11.59 seconds in HSD mice (p = 0.000). Other behavioral metrics such as line crossing, stretching, and rearing showed no significant differences (p > 0.05). These results indicate a dose-dependent reduction in anxiety-like behavior following EDTA exposure (Table 1).

Table 1: Light and Dark box test for synthetic EDTA preservative.

Parameters	Control Group	LSD (0.05 g/kg BW)	HSD (0.15 g/kg BW)	P
Line cross	6.00±2.00	8.66±2.88	6.66±0.577	0.285
Stretching	3.00±1.41	3.66±0.577	5.00±0.00	0.082
Rearing	4.00±1.41	11.00±7.93	4.00±0.00	0.133
T_D	261.5±32.9	161.00±19.97	83.66±11.59	0.000*
T_L	38.50±32.9	139.0±19.97	216.33±11.59	0.000*
Defecation	0 ± 0	0 ± 0	0 ± 0	0
Urination	0 ± 0	0 ± 0	0 ± 0	0

Hematological Parameters

As shown in Table 2, EDTA administration significantly affected select hematological markers. WBC counts increased from 1.55 ± 0.50 $\times 10^3/\mu L$ in controls to 2.80 ± 0.50 $\times 10^3/\mu L$ in the HSD group (p = 0.04), and RBC counts rose from 1.55 ± 0.50 $\times 10^6/\mu L$ to 2.80 ± 0.50 $\times 10^6/\mu L$ (p = 0.030). Hematocrit (HCT) levels nearly doubled in the LSD group (19.50 ± 5.50%) compared to the control (9.50 ± 1.2%, p = 0.022). Platelet counts also showed a significant increase, reaching 950 ± 765 $\times 10^3/\mu L$ in the HSD group versus 400.0 ± 200 in controls (p = 0.04). Other parameters such as hemoglobin, lymphocyte count, and differential percentages remained statistically unaltered (p > 0.05) (Table 2).

Table 2: Hematological Analysis of Synthetic EDTA Preservative.

Parameters	Control Group	LSD (0.05 g/kg BW)	HSD (0.15 g/kg BW)	P
WBCs	1.55±0.50	2.20±0.45	2.80±0.50	0.04*
Lym	1.3±0.98	1.5±0.55	1.75±0.91	0.852
Mon	0.05±0.07	0.00±0.00	0.1±0.00	0.085
Gran	0.200±0.14	0.1±0.00	0.15±0.07	0.453
Lym%	79.65±21.28	91.73±2.51	85.95±9.54	0.577
Mon%	4.75±4.45	1.93±0.72	3.80±1.83	0.493
Gran%	15.60±16.82	6.33±1.85	10.25±7.70	0.596
RBCs	1.55±0.5	2.20±0.45	2.80±0.50	0.030*
Hb	5.05±5.16	7.966±1.504	7.30±2.96	0.626
HCT	9.50±1.2	19.50±5.50	14.80±1.00	0.022*
MCV	51.10±13.01	51.76±2.898	52.05±2.61	0.990
MCH	21.2±16.40	23.4±4.32	28.65±10.96	0.770
MCHC	47.15±44.19	45.46±10.33	54.60±18.38	0.921
RDW	26.25±0.919	24.00±3.73	25.00±9.899	0.910
ESR	55.800±15.13	47.36±4.40	52.40±20.50	0.784
PLT	400.0±200	700.6±250	950±765	0.04*

MPV	5.90±0.848	8.233±1.73	7.25±0.92	0.289
PCT	0.251±0.19	0.50±0.34	0.64±0.63	0.658
PDW	11.85±5.44	15.30±5.78	13.7±2.545	0.770

Serological Parameters

Biochemical analysis (Table 3) demonstrated a significant reduction in LDL levels in EDTA-treated groups. LDL declined from 59.77 ± 18.86 mg/dL in the control to 27.28 ± 17.96 mg/dL in the LSD group and 20.87 ± 19.68 mg/dL in the HSD group (p = 0.032). No significant differences were observed in total cholesterol, triglycerides, HDL, total protein, creatinine, ALT, or AST levels (p > 0.05), indicating that EDTA treatment at the tested doses did not significantly impair hepatic or renal function (Table 3).

Table 3: Serological Analysis of Synthetic EDTA Preservative.

Parameters	Control Group	LSD (0.05 g/kg BW)	HSD (0.15 g/kg BW)	P
Cholesterol	360±175	236.9±106.5	247.2±47.30	0.311
Triglycerides	336±216	369.3±189.87	356.3±94.50	0.963
HDL	126.3±13.64	134.6±75.89	121.83±81.19	0.957
LDL	59.77±18.86	27.28±17.96	20.866±19.68	0.032*
Protein	6.57±3.15	5.01±2.60	5.62±1.05	0.654
Creatinine	0.772±0.64	1.77±2.12	0.426±0.33	0.425
ALT	230.2± 207.3	130.0±141.1	104.2±49.34	0.505
AST	573.3±256.94	317.2±166.4	376.6±119.8	0.160

Oxidative Stress Biomarkers

Table 4 shows that EDTA exposure induced significant oxidative stress in a dose-dependent manner. Lipid peroxidation, measured via MDA levels, increased from 1.25 ± 0.18 nmol/mg protein in controls to 2.40 ± 0.25 in LSD and 3.85 ± 0.31 in HSD mice (p = 0.002). Antioxidant enzyme activities declined with increasing EDTA dosage: SOD activity decreased from 8.75 ± 1.12 U/mg to 6.30 ± 0.98 in LSD and 4.85 ± 0.72 in HSD (p = 0.014), CAT from 52.30 ± 4.58 U/mg to 30.80 ± 3.21 in HSD (p = 0.006), and GPx from 7.90 ± 0.82 to 3.65 ± 0.47 (p = 0.009). Additionally, nitric oxide levels and protein carbonyl content were significantly elevated in treated groups, further confirming EDTA-induced oxidative imbalance and cellular stress (Table 4).

Table 4: Oxidative Stress Parameters in EDTA-Treated Mice.

Parameter	Control Group	LSD (0.05 g/kg BW)	HSD (0.15 g/kg BW)	P-value
Lipid Peroxidation (MDA) (nmol/mg protein)	3.85±0.31	2.40±0.25	1.25±0.18	0.002*
Superoxide Dismutase (SOD) (U/mg protein)	8.75±1.12	6.30±0.98	4.85±0.72	0.014*
Catalase (CAT) (U/mg protein)	52.30±4.58	41.25±3.69	30.80±3.21	0.006*
Glutathione Peroxidase (GPx) (U/mg protein)	7.90±0.82	5.10±0.69	3.65±0.47	0.009*

EDX Analysis

The results obtained from our Energy Dispersive X-ray (EDX) analysis revealed that Sodium (Na) was completely undetectable in the tissue samples from mice treated with a High Dose (HD). This finding strongly supports that our administered dose of EDTA (ethylenediaminetetraacetic acid) did not result in any detectable sodium-related deposition in the liver, kidney, and lung tissues. Since EDTA is a chelating agent that binds divalent and monovalent metal ions (including sodium), the absence of sodium signal in these organs suggests that EDTA either effectively chelated and was excreted, or it did not accumulate in the tissues to a measurable level. Consistent with previous findings by Blaurock-Busch and Busch (2025), who reported no significant tissue retention of essential elements like sodium following sodium EDTA infusion in adults, our EDX results also showed no detectable sodium in the kidney, liver, or lung tissues of HSD-treated mice, indicating effective clearance and absence of tissue accumulation (Figure 2).

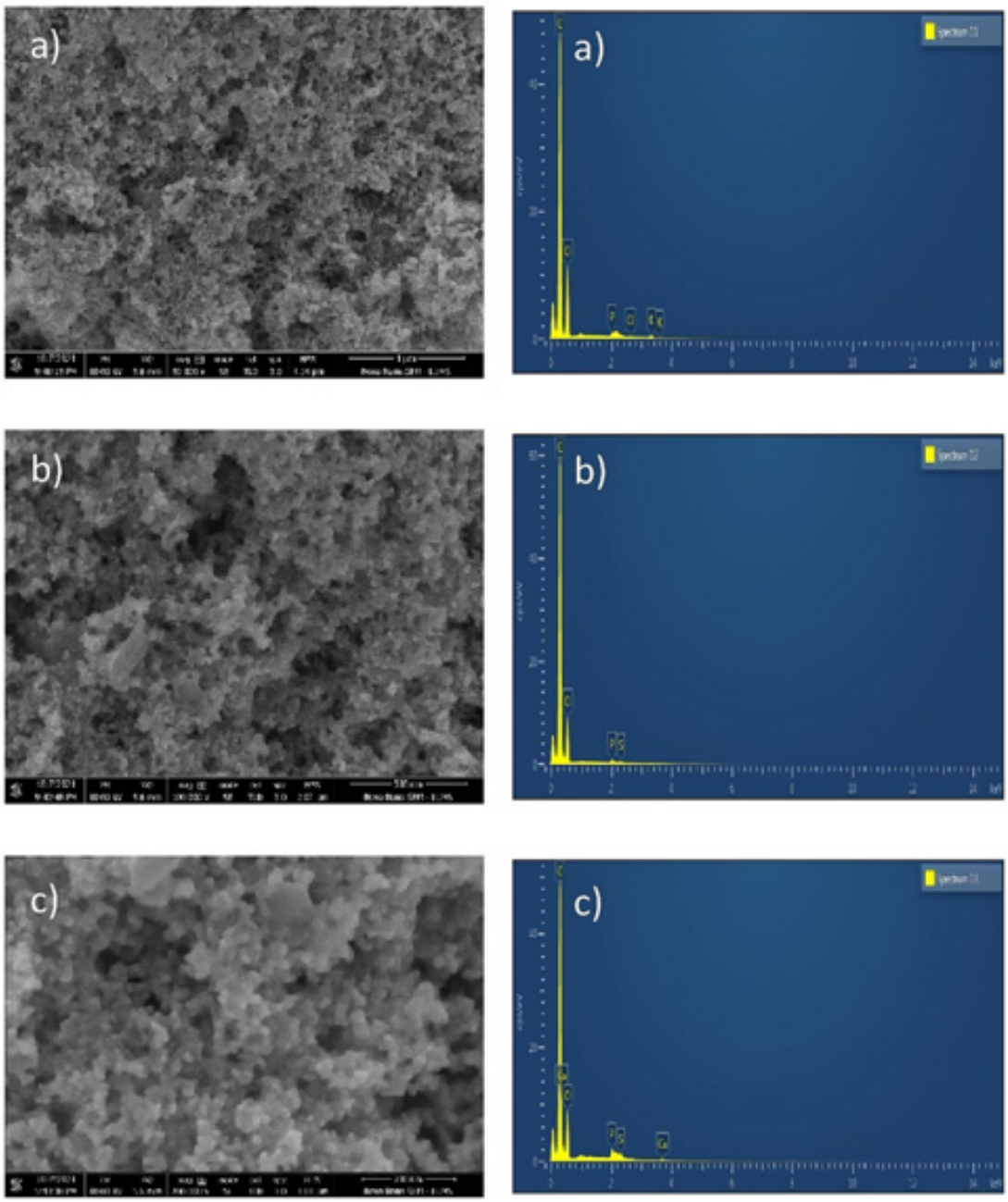


Figure 2: EDX analysis of high EDTA dose treatment.

Discussion

The observed reduction in body weight in EDTA-treated mice may be attributed to impaired nutrient absorption, metabolic disturbances, or gastrointestinal irritation caused by EDTA's chelating activity. EDTA binds essential dietary minerals such as calcium, magnesium, and zinc, which are critical for metabolic regulation and energy homeostasis. Similar findings were reported by a recent study [10], where chronic EDTA exposure resulted in weight loss in rodents due to electrolyte imbalance and reduced feed efficiency [2]. The improvement in exploratory behavior and reduced anxiety-like responses in EDTA-treated mice, as evidenced

by increased time spent in the light compartment, may be linked to alterations in neurotransmitter availability or modulation of the central nervous system via metal chelation. EDTA may reduce the burden of neurotoxic metals like lead or cadmium, indirectly enhancing synaptic function. These findings are supported by a previous study [9]. However, contrasting studies have shown neurotoxic effects of high-dose EDTA, suggesting that dose and duration critically determine the behavioral outcomes [10]. The significant increase in WBCs, RBCs, hematocrit, and platelet count in EDTA-treated mice could be attributed to mild systemic stress or immune activation. EDTA may induce a subclinical inflammatory response that stimulates hematopoietic activity in the bone marrow. Increased RBC and hematocrit levels could also result from hemoconcentration or compensatory erythropoiesis triggered by oxidative stress. The rise in platelet count may be a response to vascular oxidative injury or tissue inflammation. A study demonstrated that chronic preservative exposure altered hematological profiles in rats, including elevated RBC and WBC levels [15]. However, unlike our findings, hemoglobin levels in this study did not show significant variation, suggesting selective hematopoietic modulation depending on the chelator type or dose.

Among the biochemical parameters, only LDL levels showed a significant decrease in EDTA-treated mice. This effect might be due to EDTA's known lipid-lowering potential through inhibition of lipid peroxidation and metal-catalyzed oxidative modification of lipoproteins. Chelation of trace metals like iron and copper, which participate in LDL oxidation, may reduce circulating LDL. This finding aligns with already reported results [14]. The observations were reduced LDL and improved lipid profiles in hypercholesterolemic patients following EDTA chelation therapy [14]. Other markers of liver and kidney function (ALT, AST, creatinine) remained within normal limits, indicating that EDTA at the tested doses did not elicit overt organ toxicity. The marked elevation in malondialdehyde (MDA) levels alongside a significant reduction in antioxidant enzymes (SOD, CAT, GPx) and GSH confirms that EDTA exposure induces oxidative stress. The chelation of essential cofactors (e.g., zinc for SOD, selenium for GPx) could impair antioxidant enzyme synthesis or activity, weakening cellular defense mechanisms. These results are in agreement with the findings of [8], who demonstrated that EDTA exposure elevated oxidative stress markers and depleted antioxidant reserves in hepatic tissue [8]. The data support the hypothesis that EDTA, while a potent chelator, can disrupt the redox homeostasis by altering metal-dependent enzyme functions and promoting Reactive Oxygen Species (ROS) generation.

Conclusion

This study provides a multidimensional evaluation of EDTA's biological effects in male albino mice, extending beyond its known preservative function to assess its impact on hematological, behavioral, serological, and oxidative stress markers. While EDTA is commonly regarded as a chemically stable and effective chelating agent, our findings reveal a dose-dependent pattern of physiological and biochemical alterations. Notably, EDTA exposure significantly enhanced exploratory behavior and reduced anxiety-like responses, suggesting possible neurobehavioral modulation. Hematological analysis showed increased WBC, RBC, hematocrit, and platelet levels, indicating stimulation of hematopoiesis or systemic stress. A significant reduction in LDL cholesterol was also observed, which may reflect EDTA's influence on lipid metabolism. However, the most critical effects were observed in oxidative stress parameters. Elevated levels of lipid peroxidation (MDA), along with marked reductions in antioxidant enzymes (SOD, CAT, GPx) and Glutathione (GSH), demonstrate that EDTA compromises redox homeostasis, potentially leading to oxidative tissue damage. Despite the absence of overt toxicity in liver and kidney function markers, the findings underscore the need for cautious application of EDTA, particularly at higher concentrations, to avoid long-term oxidative and hematological disruptions. These insights call for further studies to evaluate safe exposure limits and mechanistic pathways, especially in contexts involving chronic or systemic use.

Supplementary Materials

Not applicable.

Author Contributions

All authors contributed to the design and execution of the study. All authors reviewed, edited, and approved the final version of the manuscript.

Funding

This research received no external funding.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors declare no conflict of interest.

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