Original article

Assessment of the Potential Toxic Effects of Chronic Oral Administration Calcium Disodium Ethylene Diamine Tetra-Acetate on the Liver, Kidney, Testis, and Hematological Parameters in Adult Male Albino Rats and the Role of P53 Expression

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ABSTRACT

Background: Calcium disodium Ethylene diamine tetra-acetic acid (EDTA) is a common food additive and an ingredient in cosmetic and industrial products. It's used in food to preserve flavor, color, and texture. However, like many food additives, it has become quite controversial. **Aim of the study**: assessment of the potential hepatic, renal, testicular, and hematological toxicities of chronic daily oral administration of EDTA (E385) in adult male albino rats and the involved role of p53 expression. **Materials and methods:** twenty four adult male albino rats were partitioned into two distinct groups: Control groups (Subdivided into negative and positive control groups) (8 rats each); group Ia was negative control groups received nothing, group Ib a received 0.5 ml saline via gastric gavage daily for 90 days; and group II (8rats) received EDTA by gavage at a dosage of 20 mg/kg/day for 90 days, dose equivalent to 1/100th LD50. Upon completion of the experiment, blood and tissue samples were obtained from the liver, kidney, and testis for conducting biochemical, hematological, and histopathological analyses. **Results**: Hematological analyses revealed significant decreases in white blood cells (WBCs), red blood cells (RBCs), platelet count (PT), hemoglobin level (Hb), and hematocrit value (HCT%), in the EDTA intoxicated group compared to the control groups. Biochemical tests revealed significant changes in liver and kidney functions in the EDTA intoxicated group compared to the control groups. The EDTA intoxicated group exhibited multiple histopathological changes in the liver, kidney, and testis through immunohistochemical alterations in P53 and androgen receptor expressions. **Conclusion:** Chronic oral administration of EDTA for consecutive 90 days exhibits toxic effects on the tissue of the liver, kidney, and testis through a mechanism that involves p53 expression in addition to its toxic effects on hematological parameters in male albino rats.

Keywords: EDTA, hepatic, renal, and testicular toxicity, food additive, P53, androgen receptor expression, immunohistochemistry.

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I. INTRODUCTION

Ethylene diamine tetra-acetic acid (EDTA) and its salt derivatives are commonly employed in the food industry and biomedical domains (Gil et al., 2011; Lamas et al., 2013). The Food and Drug Administration (FDA) has granted approval for the use of EDTA as a food additive in the food industry. Its purpose is to prevent oxidative deterioration by eliminating catalytic metal ions from foods and beverages, as stated by El-Naggar et al. (2021). The seed of V. fava beans is very hard; therefore, the cooking process of these beans is relatively long (Farajvand et al., 2018; El-Naggar et al., 2020). For this reason, different additives were used to decrease the time of the cooking process. Among them, some chemicals were routinely added to increase the cooking process, such as sodium bicarbonate and citric acid (Lanigan and Yamarik, 2002; Lamas et al., 2013); nowadays, EDTA is employed in the cooking procedure of the fava beans (El-Naggar et al., 2019b).

The pathogenic response to substances that damage DNA involves activation of the pro-apoptotic protein p53. P53 is one of the most important intracellular sensors of stress and damage signals (Jalili et al., 2017; Shen et al., 2023). Many apoptosis-related genes have been found to be triggered by EDTA, indicating its potential role in inducing cell death (Watt and Clarke, 1994). EDTA exhibits a diverse array of applications across multiple industries and disciplines (George and Brady, 2020). The expression of p53 is induced by a variety of stressors, oxidative stress-inducing agents, and damage pathways, with the aim of regulating cellular responses such as cell cycle arrest, repair pathways, apoptosis, and senescence (López et al., 2015).

The investigation of the potential impact of preservatives on living cells is a continuous process, given the significant increase in the utilization of synthetic preservatives in food products and the inclination of consumers towards processed food items. In many studies that investigated its toxicity, EDTA was utilized as a parenteral chelating agent for the treatment of lead and cadmium toxicity (Oloye, 2019). The widespread use of EDTA as a food additive and cooking enhancer for fava beans in contemporary society necessitates comprehensive research on the oral consumption of EDTA, necessitating further investigation.

Aim of the study: Assessment of the potential toxic effects of chronic oral administration of EDTA (E385) on the liver, kidney, testis, and hematological parameters in adult male albino rats, and assessment of the alternation of P53.

II. Materials and Methods

1. **Chemicals and reagents**

- EDTA in powder form was purchased from Sigma-Aldrich, a company based in St. Louis, MO, USA, and was subsequently dissolved in saline solution. (EDTA powder dissolved in saline) (Shandong Zhongshi International Trade Co., Ltd.).
- Biochemical kits for alanine aminotransferase, aspartate aminotransferase, urea, and creatinine were purchased from **Biodiagnostic (El-Dokky, Egypt).**

2. **Experimental animals**

A group of 24 male albino rats, with an age range of 5–6 weeks and an average weight of 200–250 g, were obtained from the Animal House of the Faculty of Medicine at Assiut University. All specimens of the Rattus genus were determined to be in a state of optimal physical condition. The experimental participants were subjected to a regulated environment with a temperature of 21.2 °C, a relative humidity range of 50–60%, and a 12-hour light/dark photoperiod. The adult rats were maintained under optimal laboratory conditions. The animals were provided with unrestricted access to both feed and water. The researchers established that the appropriate sample size for each group was 8 rats. The specimens were randomly assigned to two groups according to the following protocol: Group I (control): consisted of 16 rats subdivided into two equal subgroups (8 rats for each group); group Ia (negative control groups), to measure the fundamental values of the tested parameters, each rat got merely a usual diet and tap water, group Ib (positive control groups) received 0.5 ml of saline per rat (0.9%) (as EDTA solvent) via gastric gavage daily for 90 days.

Group II: EDTA-intoxicated male rats (8 rats) that were orally administered EDTA via gavage at a daily dose of 20 mg/kg/day (El-Naggar et al., 2020), which corresponds to 1/100th of the LD50 dissolved in 0.5ml of saline (0.9%) for 90 days. The oral LD50 of EDTA in rats was 2.0 g/kg (Lanigan and Yamarik, 2002).

Methods

Serum and tissue collection: At the end of the experiment, all rats were anesthetized with thiopental at a dose of 50 mg/kg by intraperitoneal **injection (Abdi-Azar and**

Maleki, 2014). Animals were then perfused transcardially through the left ventricle with formalin or a glutaraldehyde fixative. Specimens from the liver, kidney, and testis were taken for histopathological examinations. Blood samples were collected from the medial canthus of the eyes of rats using glass capillaries, while the rats were anesthetized with thiopental. The specimens were partitioned into two distinct segments:

Part 1: The blood was withdrawn into heparin tubes to determine the hematological parameters.

Part 2: The second blood sample was gathered in sterile and desiccated cylindrical containers and subsequently allowed to coagulate for a duration of 20 minutes at ambient temperature. The sera were separated from the blood samples through centrifugation at a rate of 4000 revolutions per minute for a duration of 15 minutes. Subsequently, the sera were preserved at a temperature of -20 ℃ until biochemical examination.

Hematological parameters

Done to estimate the total count of WBCs, RBCs, PT, Hb, and HCT% using an auto hematology analyzer (BC-3200, Mindray, China). The results were then compared with the normal values of the control group.

Biochemical Evaluation

1. **Liver function tests:** total protein, albumin serum, aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase were analyzed using colorimetric kits procured from Bio-diagnostic Company located in Cairo, Egypt.
2. **Kidney function tests:** blood urea (BUN) and serum creatinine were assayed by assaying the blood urea and serum creatinine levels through the colorimetric method, utilizing kits procured from the Bio-diagnostic Company based in Cairo, Egypt.

Histopathological examination

For the histopathological study, biopsies were taken from rats' control groups (Ia and Ib) and EDTA-intoxicated (Group II) liver, kidney, and testis. Biopsies were processed for examination in the following ways:

1. **Light microscope (LM) examination:** The liver, kidney, and testis were extracted and subjected to light microscopy processing. A few paraffin sections were stained with:

I. **Hematoxylin and eosin (H&E):** For a comprehensive histological analysis, the methodology outlined by **(Bancroft and Gamble, 2008) may be emplo**yed.

II. **Periodic Acid-Schiff (PAS):** Some testis sections were stained to detect polysaccharides in the tunica albuginea and the basement membrane around seminiferous tubules (Bancroft and Gamble, 2008).

Immuno-histochemical methods:

I. **p53:** immuno-histochemical (staining the nucleus) of liver, kidney, and testis paraffin sections with a primary antibody targeting p53 (Rabbit monoclonal, Clone SP5, Neomarkers, USA) to illustrate the presence of apoptotic cells. The technique of avidin-biotin peroxidase was implemented in accordance with the methodology outlined by O'Callaghan and Jensen (1992). The sections were subjected to incubation with the primary antibody p53, which was diluted at a ratio of 1:100 in phosphate buffer solution (PBS) and allowed to incubate overnight at ambient temperature. The entity in question exhibits a nuclear expression. In order to perform negative control staining, certain sections were subjected to incubation with phosphate-buffered saline (PBS) in lieu of the primary antibody. The sections under investigation did not exhibit any detectable immunoreactivity. Positive control staining involves the utilization of sections of breast carcinoma as positive controls.

II. **Androgen receptors:** immuno-histochemical staining of testis paraffin sections was conducted using polyclonal antibodies for androgen receptors (Cat. No. MA1-150, Thermo Fisher Scientific Co., USA). The O'Callaghan and Jensen (1992) protocol for the avidin-biotin peroxidase technique was employed. The sections were subjected to incubation with the primary antibody androgen receptor, which was diluted to a ratio of 1:100 in phosphate buffer solution (PBS) and were allowed to incubate overnight at room temperature. The object under consideration exhibits a nuclear expression. To perform negative control staining, certain sections were subjected to incubation with phosphate-buffered saline (PBS) in lieu of the primary antibody. The sections under investigation did not exhibit any discernible immunoreactivity. Positive control staining involves utilizing sections of prostatic cancer as positive controls.

Morphometric analysis:

The morphometric analyses were conducted utilizing Image J, an open-source image processing software based on Java. The study involved the measurement of required parameters in five distinct fields, each comprising five non-overlapping sections, with

five rats being selected from each group. The parameters that were measured are as follows:

I. The number of p53 positive-immunoassayed cells was counted in rat livers, kidneys, and testis in groups Ia, Ib, and II.

II. Height of cells in seminiferous tubules (by image J software program) (height of seminiferous tubules is an indicator of the number of rows of spermatogenic cells) in the H&E-stained sections of the testes in control groups (Ia, Ib) and group II. Epithelial height was quantified in micrometers for 50 tubules selected at random from testicular sections of mice in each experimental group. The measurement of epithelial height was conducted by utilizing the arbitrary distance method and observing sections with a 40X objective lens. Two points along the same axis of each tubule were assessed. The epithelial height was determined by calculating the average of the two readings.

III. The percentage of positive PAS staining reactions in the testes of rats belonging to control groups (Ia, Ib) and group II.

IV. The area percentage of androgen receptor immune-positive reactions in the testis of rats belonging to control groups (Ia, Ib) and group II.

Statistical analysis

Data were expressed as means ± SD. One-way ANOVA was used to assess the statistical significance of differences between the experimental groups. A p-value less than 0.05 was regarded to be statistically significant. All statistical analysis was performed using SPSS 26.0.

Ethical consideration:

The experiments were conducted in adherence to established protocols and international guidelines for the proper care and use of laboratory animals in research. Approval for the experiments was granted by the Ethical Committee of the Faculty of Medicine at Assiut University in Egypt with Ethical Number "04-2024-300412".

III. RESULTS

According to biochemical analysis and histopathological findings, EDTA intoxication resulted in various degenerative morphological changes in rats' liver, kidneys, and testis.

Results of biochemical analysis

- **Liver function and kidney function tests:**

The findings indicated a notable disparity in albumin, ALT, alkaline phosphatase, and serum urea levels between control groups (Ia, Ib) and group II. Conversely, it demonstrates that there is no notable distinction between control groups

(Ia, Ib) and group II regards to total protein, total bilirubin, direct bilirubin, AST, GGT, or serum creatinine **(Table 1)**.

- **Hematological analysis:**

There were highly significant differences observed in the hematological parameters between the control groups (Ia, Ib) and group II concerning a significant decrease in the total count of WBCs (p<0.001**), Hb (p 0.001**), total count of RBCs (p 0.001**), the number of platelets (p<0.001**), and hematocrit value (p 0.009**) **(Table 2)**.

Results of the histopathological examination:

The histopathological picture of both the negative and positive groups was similar and gave the same normal architecture, so we used the negative control group as a standard reference in comparison with the EDTA-intoxicated group.

A-Liver:

1. Light microscopic:

The histological examination of the liver tissue samples obtained from the control group using light microscopy and H&E staining revealed the presence of a typical hepatic architecture characterized by the presence of a central vein and radiating plates of hepatocytes that were separated by blood sinusoids. The polygonal shape of the hepatocytes is accompanied by vesicular central nuclei and acidophilic cytoplasm, (as depicted in **Figure 1a)**. EDTA was administered by group II, and subsequent examination of liver specimens revealed congested central veins that were significantly dilated and filled with extravasated red blood cells, (as depicted in **Figure 1b)**. The observed hepatocytes exhibited hydropic degeneration characterized by vacuolated cytoplasm and pyknotic nuclei, (as depicted in **Figure 1c)**. Furthermore, the blood sinusoids exhibit distortion, (as depicted in **Figure 1d)**.

2. Immunohistochemistry:

The hepatocytes of the control group exhibited immuno-negative expression of p53 in the liver specimens of rats. **Figure (1e)** illustrates a notable increase in the manifestation of p53 within hepatocytes in EDTA-intoxicated group. The statistical results demonstrate a significant increase in the number of hepatocytes positive for p53 in the EDTA-intoxicated group. This is supported by a P-value of 0.001 (as depicted in **Figure 1f)**.

B- Kidney:

1. Light microscopic:

H&E-stained sections of the kidneys of rats belonging to the control group exhibited a typical renal cortex structure comprising proximal and distal convoluted tubules as well as Bowman's corpuscles, (as depicted in **Figure 2a)**. Upon administration of EDTA by group II, the specimens of the kidneys exhibited vacuolar

degeneration in both proximal and distal convoluted tubules (as depicted in **Figure 2b**), along with renal blood vessel congestion (as shown in **Figure 2c)** and degeneration and destruction of Bowman's corpuscles (as illustrated in **Figure 2d**).

2. Immunohistochemistry:

The rats' kidney specimens showed few p53 immuno-positive renal cells in the control group**.** Numerous p53 positive renal cells were found in the EDTA intoxicated group **(Figure 2e).** Statistical results show a significant increase in the number of p53 positive renal cells in the EDTA intoxicated group (P-value < 0.001) **(Figure 2f).**

C- Testis:

1. Light microscopic:

The histological analysis of paraffin sections of rats' testis from the control group, using H&E staining, reveals the presence of multiple seminiferous tubules that are surrounded by a basement membrane. The tubules were observed to be lined with spermatogenic cells at different stages of maturation, which included Sertoli cells. Spermatozoa are observed within the lumen of the seminiferous tubules (as depicted in **Figure 3a)**.

Within the second group, it was observed that EDTA administration resulted in the rupture of numerous seminiferous tubules, (as depicted in **Figures 3b, 3c)**. The thickness of cells involved in spermatogenesis exhibits a reduction (as depicted in **Figures 3b, 3c, and 3d)**. The cells involved in spermatogenesis undergo dissociation from both the basement membrane and intercellular connections, (as depicted in **Figures 3c, 3d)**. The cytoplasm of the interstitial Leydig cells is characterized by the presence of vacuoles and residual nuclei, (as depicted in **Figure 3e)**. The PAS-stained paraffin sections of the testis in the control group exhibited affirmative responses in the basement membranes that encircle the seminiferous tubules and tunica albuginea (as depicted in **Figure 4a**). In contrast, the EDTA-intoxicated group displayed a thickening of the tunica albuginea and basement membrane (as illustrated in **Figure 4b**).

2. Immunohistochemistry:

I. **p53**

The testicular specimens of rats in the control group exhibited a lack of p53 expression in spermatogenic cells, (as depicted in **Figure 5a)**. Conversely, the rats in the EDTA-intoxicated group were subjected to p53-positive spermatogenic cells, (as illustrated in **Figure 5b)**. The statistical findings indicate a noteworthy rise in the count of p53-positive spermatogenic cells in the EDTA-intoxicated group with a P-value of 0.001 (as illustrated in **Figure 5d**).

II. **Androgen Receptor**

The specimens of testis from rats exhibited affirmative immune expression of androgen receptor in interstitial Leydig cells in the control

group. The EDTA-intoxicated group showed a decrease in immune expression of androgen receptors in interstitial Leydig cells, (as depicted in **Figure 5c)**. The statistical findings indicate a noteworthy reduction in the proportion of androgen receptor positive immunoreactivity in the testis of group II (P-value < 0.001) **(Figure 5e).**

Table 1: Biochemical analysis of liver function and kidney function among studied groups by ANOVA one way and LSD tests

Parameters	Group Ia Mean±SD (n=8)	Group Ib Mean±SD (n=8)	Group II Mean±SD (n=8)	P. value	P1(1a & Ib)	P2(Ia & II)	P3(Ib & II)
Total protein	7.35±0.65	7.31±0.05	7.37±0.04	0.0860	0.092	0.674	0.034*
Albumin	2.15±0.68	1.94±0.17	1.36±0.16	<0.001**	0.916	<0.001**	<0.001**
AST	71.49±21.74	86.26±14.06	110.75±12.84	0.002**	0.206	0.002**	0.003**
ALT	35.25±9.04	33.66±6.24	61.1±9.47	<0.001**	0.527	0.002**	<0.001**
ALP	86.09±24.03	85.76±12.33	109.27±3.76	<0.001**	0.292	0.001**	<0.001**
BUN	7.11±1.04	7.48±0.69	8.98±0.22	<0.001**	0.460	0.001**	<0.001**
Serum Creatinine	44.13±14.05	43±8.85	55.26±16.56	0.1010	0.398	0.206	0.035*

n: number of rats; *: Statistically significant (p ≤ 0.05); P1: significance in relation to group Ia; P2: significance in relation to group Ib; P3: significance in relation to group II; AST: aspartate aminotransferase; ALT : alanine aminotransferase; ALP: alkaline phosphatase; BUN: blood urea. Data are expressed as mean± SD. Data were analyzed by one-way ANOVA with pairwise comparison with the use of LSD.

Table 2: Hematological parameters Relationship among studied groups by ANOVA one way and LSD tests

Parameters	Group Ia Mean±SD (n=8)	Group Ib Mean±SD (n=8)	Group II Mean±SD (n=8)	P. value	P1(1a & Ib)	P2(Ia & II)	P3(Ib & II)
WBC (103 /µl)	11.28±0.24	11.26±0.25	9.01±0.31	<0.001**	0.915	<0.001**	<0.001**
Hb (%)	13.74±0.86	13.58±0.47	11.71±0.84	0.001**	0.457	0.003**	0.002**
RBCs (106 /mL)	9.41±0.48	9.05±0.36	8.3±0.62	0.001**	0.113	0.001**	0.008**
Platelets (103 /µl)	979.37±32.28	978±31.45	760.35±24.66	<0.001**	0.793	<0.001**	<0.001**
HCT%	43.52±2.43	43.4±0.22	37.84±3.51	0.007**	0.140	0.009**	0.011*

n: number of rats; *: statistically significant (p ≤ 0.05); P1: significance in relation to group Ia; P2: significance in relation to group Ib ; P3: significance in relation to group II. WBCs: white blood cells; Hb: hemoglobin; RBCs: red blood cells; HCT%: hematocrit value. Data are expressed as mean± SD. Data were analyzed by one-way ANOVA with pairwise comparison with the use of LSD.

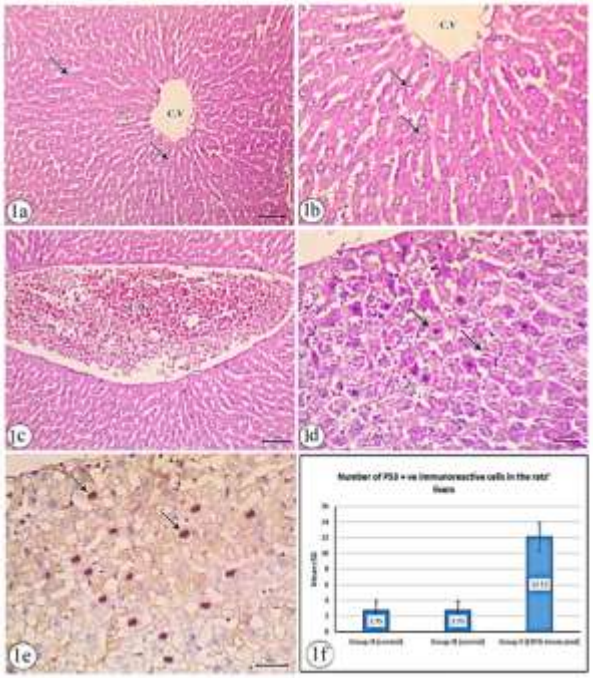

Figure (1): A photomicrograph of liver sections stained by H&E showing: (1a); control group revealed hepatocytes with central round vesicular nuclei (black arrow), radiating from the central vein (C.V), and separated by blood sinusoids (x100 and x200). (1b),(1c) & (1d) EDTA-intoxicated group where (1b); congested dilated central vein (C.V) filled with RBCs (x200 and x400), (1c) cords of hepatocytes with hydropic degeneration, pykontic nuclei, and vacuolated cytoplasm, and (1d) the blood sinusoids are distorted (black arrow) (x400). (1e); EDTA-intoxicated group with positive immuno-expression of p53 in hepatocytes (black arrow) (x 400). (1f) A histogram showing a significant increase in the mean number of p53 immuno-positive hepatocytes in the EDTA-intoxicated group (P-value < 0.001).

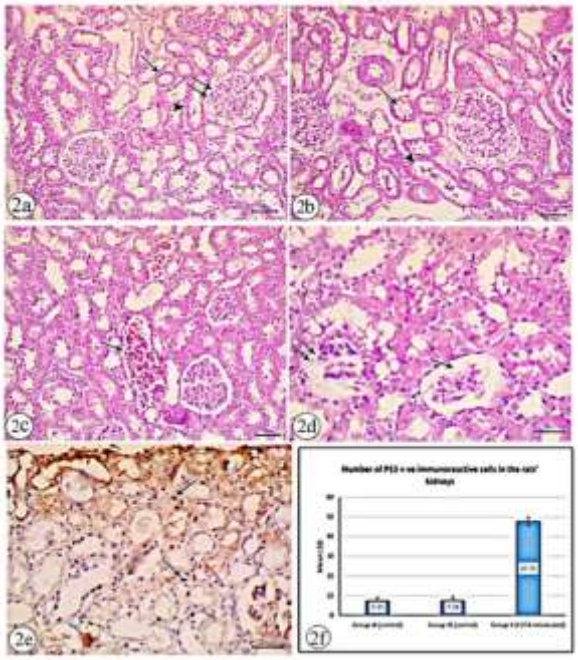

Figure (2): A photomicrograph of kidney sections stained by H&E showing: (2a); control group revealed renal cortex containing proximal convoluted tubules (black arrow), distal convoluted tubules (black arrowhead), and Bowman's corpuscles (two black arrows) (x200 and 400). (2b), (2c) & (2d) EDTA-intoxicated group where (2b); vacuolar degeneration of epithelial lining proximal convoluted renal tubules (black arrow), distal convoluted tubules (black arrowhead) (x200), (2c) congestion of renal blood vessels (black arrow) (x200), and (2d) degeneration of the glomerular tuft with widening of Bowman's space (black arrow). Another Bowman's corpuscle (two black arrows) shows complete degeneration of glomerular tuft and endothelial lining (x400). (2e); EDTA-intoxicated group with numerous p53 positive renal cells (black arrow) (x400). (2f) A histogram showing a significant increase in the mean number of p53 immuno-positive renal cells in the EDTA-intoxicated group (P-value < 0.001).

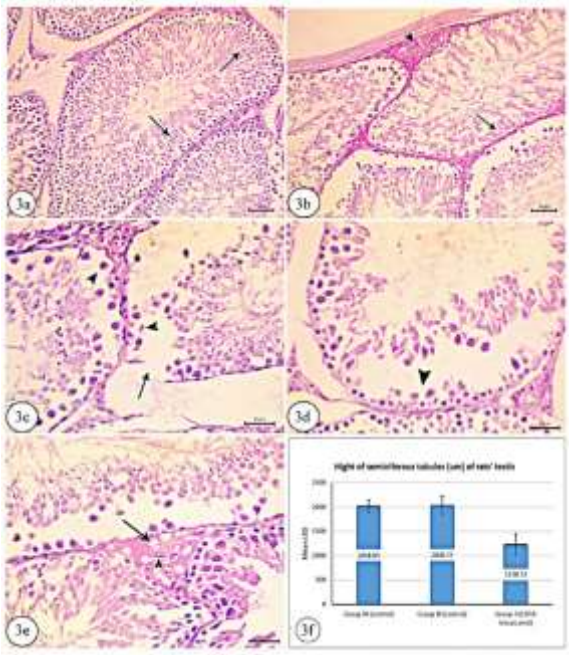

Figure (3): A photomicrograph of testis sections stained by H&E showing: (3a): control group; seminiferous tubule lined by several layers of spermatogenic cells (black arrow) (x200). (3b); two adjacent seminiferous tubules surrounded by a basement membrane (black arrow) and lined by several rows of spermatogenic cells (black arrowhead) and Sertoli cells (curved arrow) in-between. Numerous grouped interstitial Leydig cells (two black arrows) are present in between the seminiferous tubules and have round vesicular nuclei (x400). (3c), (3d) & (3e) EDTA-intoxicated group where (3c); seminiferous tubules with a reduction in the layers of spermatogenic cells (black arrow). Note: the congested blood vessel (black arrowhead) (x200), and (3d and 3e) disrupted basement membrane (black arrow), and the spermatogenic cells (black arrowhead) are markedly reduced and dissociated from the basement membrane and from each other (x400), and (3e) interstitial Leydig cells with vacuolated cytoplasm (black arrow) and densely stained nuclear remnants (black arrowhead) (x400). (3f) A histogram showing a significant reduction in the mean height of seminiferous tubules in the EDTA-intoxicated group (P value < 0.001**).

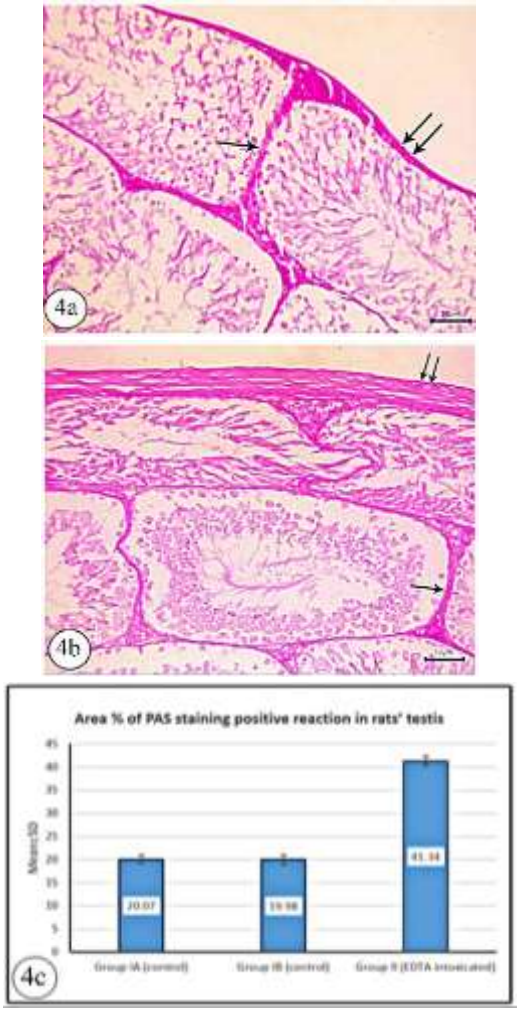

Figure (4): A photomicrograph of testis sections stained by PAS showing: (4a); revealed PAS positive reaction in the basement membrane around seminiferous tubules (black arrow) and tunica albuginea (two black arrows) (x200). (4b) EDTA-intoxicated group where a strong PAS positive reaction with thickened tunica albuginea (two black arrow) and basement membrane (black arrow) (x200). (4c) A histogram showing a significant increase in the area% of PAS positive reaction in the EDTA-intoxicated group (P value < 0.001**).

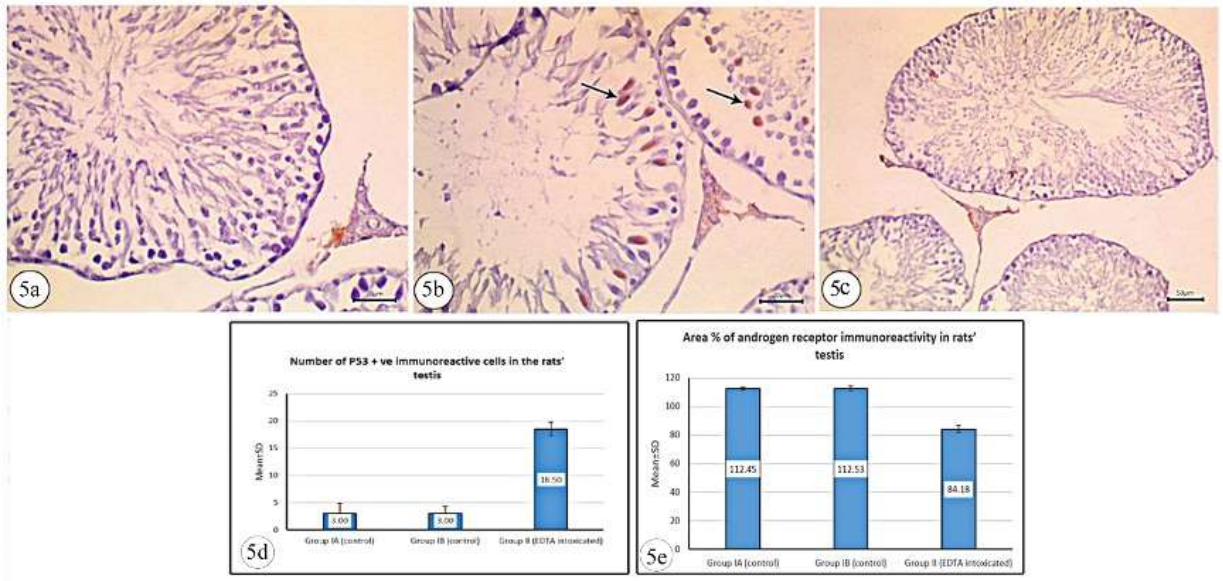

Figure (5): A photomicrograph of testis sections with immunohistochemical staining showing: (**5a**); control group revealed negative immune expression of p53 in spermatogenic cells (x400). (5b) & (5c) EDTA-intoxicated group where (5b); p53 positive spermatogenic cells (black arrow) (x 400), (5c) negative expression of androgen receptor in interstitial Leydig cells (x200). (5d) A histogram showing a significant increase in the mean number of p53 immuno-positive spermatogenic cells in the EDTA-intoxicated group (P-value < 0.001). (5e) A histogram showing a significant decrease in the area percentage of androgen receptor positive immunoreactivity in interstitial Leydig cells in the EDTA-intoxicated group (P-value < 0.001).

IV. DISCUSSION

EDTA and its salt derivatives have been widely used in the field of the food industry. Prolonged oral administration of EDTA could be toxic, depending on the administration route and the doses **(Ramadan et al., 2016)**. The objectives of this study were to assess the potential toxic effects of chronic oral administration of EDTA (E385) on the liver, kidney, testis, and hematological parameters in adult male albino rats and assess the alternation of P53 expression.

In the present study, the ALT, AST, urea, and creatinine biomarkers increased after chronic EDTA administration, with altered biochemical and physiological features of liver and kidney tissues. These findings were in agreement with **El-Naggar et al. (2020),** who reported that administration of EDTA increased liver and kidney biomarkers. **Abou-Zaid et al. (2021)** demonstrated that the livers of male rats receiving CaNa2EDTA orally had a substantially high level of oxidative stress, which was the cause of the histological alterations. In agreement with **El-Naggar et al. (2020)**, who reported that the hepatic and renal architectures were disorganized with some hemorrhagic manifestations in the livers and kidneys of mice, these results demonstrate that EDTA is harmful in mice under the conditions of this study, and the potentially harmful effects in humans support restricting its use.

The toxic effects of EDTA are due to its metal ion chelation capacity, which alters ion distribution in tissues and precludes metalo-enzyme activities. Absorption of EDTA into the blood stream is a crucial step in producing its toxic effects (El-Naggar et al., 2020). The control group's hematological values were within the typical ranges for rats that are mentioned in the literature (Etim et al., 2014; Jacob Filho et al., 2018). The considerable reduction in hemoglobin concentration and lower RBC count point to the beginning of an anemic condition in the EDTA-intoxicated group of rats. In both people and animals, exposure to chemicals and toxins can affect or harm the hematological profile (Gbore et al., 2020). This finding could be due to the toxic effect of EDTA byproducts on the bone marrow or spleen. A previous study showed that EDTA could alters hematological parameters post chronic administration (El-Naggar et al., 2019a; El-Naggar and El-Said, 2020).

Administration of EDTA (10 mg/Kg) for 90 days alter the differential leucocytes. This finding could be due to the toxic effect of EDTA byproducts on the bone marrow or spleen (El-Naggar et al., 2021). The observed decrease in platelet counts in EDTA-intoxicated group could be due to EDTA binding to platelets, which might induce agglutination via the stabilization of platelet membranes (El-Naggar et al., 2020). The present data were also in agreement with a previous study on the toxicity of disodium EDTA in mice (Chaudhary et al., 2016). Moreover, EDTA lowers platelet counts, suggesting that it causes bone marrow depression and lowers blood cell counts overall, which is consistent with Lee et al. (2013) findings. El-Naggar et al. (2020) reported that the disorganized hepatic and real architectures in EDTA intoxicated groups confirm the toxic effects of EDTA on mice livers and these harmful effects due to bean cooking media and the presence of EDTA in the cooking media.

In experimental animals, EDTA administration promoted histological alterations. The present study indicated that there were prominent histopathological alterations in the liver of mice administered EDTA, and these results are in agreement with Ramadan et al. (2016) and El-Naggar et al. (2020), who stated that EDTA chronic toxicity on the liver appears as disorganization of hepatic architecture, cytoplasmic vacuolation and degeneration, congestion of blood vessels, cellular infiltration, necrotic areas, and apoptotic bodies.

The current study discusses a research inquiry pertaining to the toxicity mechanism of EDTA as a food supplement. The study findings demonstrate a noteworthy upsurge in the expression of p53, which is one of the apoptotic pathways, in livers that have been subjected to EDTA intoxication (Tosun et al., 2014). The finding is corroborated by Guo et al. (2013), whose research demonstrated that glycyrrhizic acid mitigated hepatocyte apoptosis in rats through a pathway mediated by p53. Apoptosis is

a process that plays a role in the progression of liver fibrosis, as noted by Guo et al. (2013).

According to Kodama et al. (2011), the activation of endogenous p53 in hepatocytes of double-minute knockout mice leads to the occurrence of spontaneous liver fibrosis. The present study illustrates the deleterious impact of elevated dosages of EDTA on the hepatic system of male rodents and establishes a correlation between the quantity of EDTA administered and the extent of histopathological modifications observed in the liver.

According to Kim et al. (2015), the administration of EDTA led to a marked increase in blood urea nitrogen and serum creatinine levels, indicating the occurrence of renal toxicity. The histopathological analysis reveals the degeneration of Bowman's corpuscle, congestion of renal blood vessels, and vacuolar degeneration of the proximal and distal convoluted tubules. In agreement with El-Naggar et al. (2020), histological studies of kidney sections of mice administered with EDTA revealed disorganized glomeruli and atrophied ones with eroded walls of Bowman’s capsules.

The present histopathological findings were in line with previous studies that reported some histopathological changes of the kidney sections, showing mild to moderate renal changes with swelling in the focal subcapsular and glomerular loops proliferation. Previous studies determined that EDTA is a hazardous chemical with the potential to penetrate biological membranes and tissue stroma. Reuber and Schmieler (1962), as well as Chaudhary et al. (2016), conducted studies that demonstrated the presence of hydropic alterations in the kidney, as well as glomerular loop multiplication and focal subcapsular edema, which support the current findings.

Consistent with the results reported by Homsi et al. (2011), the present study reveals a significant elevation in p53 immunoreactivity in kidneys exposed to EDTA toxicity. The year 2010 marked the discovery that the acute damage caused by glycerol-induced oxidative stress led to an upregulation of p53 expression in renal tubules. According to Tosun et al. (2014), the administration of EDTA results in cellular DNA damage and death, as evidenced by the upregulation of the p53 gene, which is indicative of DNA damage in cells. In individuals with EDTA-induced impairment, the seminiferous tubules in the testis exhibited ruptures, a reduction in the thickness of spermatogenic cells, and a loss of connection to both the basement membrane and to neighboring cells. Additionally, interstitial Leydig cells displayed degenerative alterations. Ramadan et al. (2016) reported the observation of foamy seminiferous tubule lumens, slugs of spermatogenic cells from the basement membrane, severe testicular tissue degeneration, and severe necrosis in both germ cells and interstitial cells subsequent to the administration of EDTA.

The present study reveals that exposure to EDTA toxicity results in a notable increase in expression of tunica albuginea and basement membrane, as evidenced by a positive PAS reaction and thickening of these structures in the testis. El Gharabawy et al. (2019) reported robust PAS-positive responses in the thickened and irregular basement membrane, interstitium, and thickened vascular walls in the group intoxicated with cyclophosphamide, which aligns with the current findings.

According to Ramadan et al. (2016), the ingestion of EDTA at concentrations ranging from 0.5 to 5 g/kg of food resulted in a dose-dependent reduction in the fertility of male rats, as EDTA was toxic for meiotic cell proliferation even at low concentrations, and its toxicity increased in a dose-dependent manner. Prior research has suggested that the ingestion of oral EDTA in animals could potentially result in negative impacts on their reproductive and developmental functions (Khalil et al., 2008).

Banerjee et al. (2016) conducted a study that demonstrated a noteworthy augmentation in the phosphorylation of p53, as well as a subsequent rise in both p53 protein and mRNA levels in germ cells, because of exposure to benzopyrene. Androgen is a vital factor in maintaining spermatogenesis in the testis and facilitating the maturation of spermatozoa in the epididymis. The physiological effects of androgen are facilitated by the androgen receptor (AR), which is regulated by levels of androgen.

Immunohistochemical analysis revealed the presence of androgen receptor (AR) in the nuclear compartments of Sertoli cells, peritubular myoid cells, and Leydig cells within the testicular tissue. The results of this investigation indicate that the consumption of EDTA led to a decline in androgen concentrations, which consequently caused a decrease in the production of sperm cells. The observed decline in androgen receptor (AR) expression in the testes of rats exposed to EDTA provides support for this claim. The inadequacy in the process of sperm cell production was confirmed through a comparison of the height of seminiferous tubules between the group that was impaired by EDTA and the control group's P-value.

V. Conclusion & Recommendations

Even at low doses, EDTA is harmful to male rats' livers, kidneys, testis, and hematological parameters in the study's settings, despite its safe usage in chelating therapy and the food business. A plan for the proper use and management of EDTA in the public domain must be developed. Further research is needed to evaluate the danger to people under actual consumption situations, investigate other toxic effects of EDTA on human health.

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تقييم التأثيرات السامة المحتملة للتعرض المزمن عن طريق الفم لثنائي صوديوم الكالسيوم إيثيلين دياميين رباعي أسيتات على الكبد والكلى والخصية و الدم في ذكور الجرذان البيضاء البالغة ودور تعبير P 53

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الخلفية: ثنائي صوديوم الكالسيوم EDTA هو مادة مضافة غذائية شائعة ومكون في مستحضرات التجميل والمنتجات الصناعية. يتم استخدامه في الطعام للحفاظ على النكهة واللون والملمس. ومع ذلك، مثل العديد من المضافات الغذائية، فقد أصبح استخدامها مثيرا للجدل إلى حد كبير.

الهدف من الدراسة: تقييم السمية الكبدية والكلوية والخصوية والدموية المحتملة عند تناول (E385) (EDTA) عن طريق الفم يوميًا في ذكور الجرذان البيضاء البالغة والدور المتضمن في تعبير p53.

الطريقة: تم اجراء البحث على اربع وعشرون من الجرذان البيضاء البالغة من الذكور، وقد قسموا الى مجموعتين متساويتين ، المجموعة الاولى حيث قسمت الجرذان الى فئتين: I أ كانت المجموعة الضابطة السالبة ، المجموعة I ب الضابطة الموجبة اعطائها 0.5 ملليتر من محلول الملح عن طريق الفم لمدة 12 أسبوع، المجموعة II تلقت EDTA بجرعة 20 ملجم/كجم/يوم لمدة 90 يومًا. عند الانتهاء من التجربة، تم سحب عينات الدم والأنسجة تم الحصول عليها من الكبد والكلى والخصية لإجراء التحليلات البيوكيميائية والدموية والنسيجية.

النتائج: كشفت تحاليل الدم عن انخفاض ملحوظ في عدد كرات الدم البيضاء، كرات الدم الحمراء، وعدد الصفائح الدموية، ومستوى الهيموجلوبين، وقيمة الهيماتوكريت في المجموعة المعالجة بـ EDTA مقارنة بمجموعات المقارنة.. كشفت الاختبارات البيوكيميائية عن تغيرات معنوية في وظائف الكبد والكلى في المجموعة المعالجة بـ EDTA مقارنة بمجموعات المقارنة.. أظهرت المجموعة المعالجة بـ EDTA تغيرات نسيجية متعددة في الكبد والكلى والخصية من خلال التغيرات الكيميائية المناعية في تعبيرات مستقبلات الاندروجين P53.

الخلاصة: خلصت الدراسة إلى أن تناول EDTA عن طريق الفم بشكل مزمن لمدة 90 يومًا متتالية يظهر تأثيرات سمية على أنسجة الكبد والكلى والخصية من خلال آلية تتضمن تعبير p53 بالإضافة إلى آثاره السامة على مؤشرات الدم في ذكور الجرذان البيضاء.

التوصيات: يوصي بوضع بروتوكول للاستخدام السليم الامن للاديتا بوجه عام واجراء المزيد من الابحاث والدراسات على الانسان لتقييم المخاطر التي يتعرض لها البشر في حالات الاستهلاك الفعلي للاديتا، ودراسة وتحليل التأثيرات السامة الأخرى للاديتاعلى صحة الانسان.