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The Ameliorative Effects of Broccoli Extract on Lead-Induced Reproductive Toxicity and DNA Damage in Pre-Pubertal Rats

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Abstract

Lead toxicity poses significant risks to reproductive health, primarily through oxidative stress and endocrine disruption. This study evaluates the protective effects of ethanolic broccoli extract against lead-induced reproductive toxicity in prepubertal male Wistar rats. The study involved five groups: Control, Lead Acetate (LA), Lead Acetate + Broccoli extract (LA + Broc), Lead Acetate + Vitamin E (LA + Vit E) and Broccoli extract (Broc). Results revealed statistically significant differences across all parameters ($p < 0.05$). The lead group exhibited severe reductions in testis weight, antioxidant enzyme levels (GPX, SOD, GSH, CAT), oxidative stress parameter (LPO), reproductive hormone levels (testosterone, FSH, LH) and sperm quality, along with elevated lipid peroxidation (LPO) and DNA damage. Histopathological analysis revealed extensive degeneration of seminiferous tubules and reduced cellular proliferation (Ki67-positive cells).

Conversely, the Broc group demonstrated substantial protective effects including normalization of testis weight, enhancement of antioxidant defenses, restoration of hormone levels and improved sperm parameters. Broccoli extract also mitigated histopathological alterations and significantly reduced DNA tail length, a marker of genotoxicity. The LA + Broc and LA + Vit E groups exhibited partial recovery, with broccoli extract showing superior efficacy over vitamin E, likely due to its bioactive compounds such as sulforaphane. Histomorphometric improvements in seminiferous tubule diameter, luminal diameter and epithelial thickness further underscored the protective role of broccoli extract.

These findings highlight the potential of broccoli extract as a natural therapeutic agent for mitigating lead-induced reproductive toxicity, offering insights into its antioxidant and anti-inflammatory mechanisms. Further studies are warranted to explore their applicability in the clinical settings.

Keywords: Broccoli extract, Lead-induced toxicity, Oxidative stress, Reproductive toxicity, Antioxidant enzymes, Pre-pubertal Wistar rats.

Introduction

Environmental and industrial contamination with heavy metals such as lead remains a significant public health concern, particularly due to its deleterious effects on various biological systems. Lead exposure is associated with severe toxicity, especially in vulnerable populations such as children and adolescents where it interferes with developmental processes including reproductive health and genomic stability[10]. Lead-induced toxicity has been linked to oxidative stress, disruption of endocrine function and damage to the hypothalamic-pituitary-gonadal axis, leading to adverse reproductive outcomes[34]. Experimental studies have also demonstrated the potential of lead to cause DNA damage, highlighting the urgency of investigating effective protective agents against its toxic effects.

Natural products, particularly those with high antioxidant content, have gained attention as potential mitigators of heavy metal toxicity. Among these, broccoli (*Brassica oleracea var. italica Plenck*) has shown promise due to its abundant bioactive compounds including glucosinolates, flavonoids and vitamins C and E which exhibit significant antioxidant and anti-inflammatory properties[13]. Broccoli has been extensively studied for its health-promoting effects including detoxification of harmful compounds, modulation of oxidative stress and prevention of chronic diseases[35]. However, its potential in mitigating lead-induced reproductive toxicity and DNA damage has been relatively unexplored, particularly in pre-pubertal populations.

Oxidative stress is a central mechanism in lead-induced toxicity, characterized by an imbalance between reactive oxygen species (ROS) production and the antioxidant defense system[8]. Excessive ROS levels can result in lipid peroxidation, protein degradation and DNA damage which collectively compromise cellular integrity and function.

Lead's ability to generate oxidative stress is further exacerbated by its interference with enzymatic and non-enzymatic antioxidants, thereby weakening the body's natural defenses[29]. This underlines the importance of identifying antioxidant-rich agents that can replenish

endogenous defenses and protect against lead-induced damage.

Broccoli's high levels of sulforaphane, a potent antioxidant derived from glucosinolates, contribute to its protective effects against oxidative damage[38]. Sulforaphane activates the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway, enhancing the expression of detoxifying enzymes such as glutathione S-transferases and heme oxygenase-1 which play critical roles in mitigating oxidative stress[28]. These properties make broccoli extract a compelling candidate for investigating its efficacy in ameliorating lead-induced toxicity.

Reproductive toxicity caused by lead exposure is multifaceted, involving disruption of spermatogenesis, hormonal imbalances and damage to reproductive organs[11]. Studies in animal models have demonstrated significant reductions in testosterone levels, alterations in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and histopathological changes in testicular tissue upon lead exposure[29]. DNA damage, a hallmark of genotoxicity, further complicates the scenario, as it has long-term implications for reproductive health and offspring viability[34].

Preclinical studies have shown that natural antioxidants like broccoli extract can mitigate lead's toxic effects by reducing oxidative stress and preserving normal reproductive functions[13]. The bioactive components in broccoli not only scavenge free radicals but also modulate inflammatory pathways, thereby preventing tissue damage and promoting repair[35]. These findings suggest that broccoli extract may serve as an effective therapeutic intervention for counteracting lead-induced reproductive toxicity and DNA damage in pre-pubertal rats. The current study aims to evaluate the ameliorative effects of broccoli extract on lead-induced reproductive toxicity and DNA damage in pre-pubertal rats.

Material and Methods

Chemicals: All reagents and chemicals used for this study were of analytical grade quality obtained from Sigma-Aldrich Chemical Company (USA) and Sisco Research Laboratories (India).

Experimental Animals: The study was conducted with 30 male pre-pubertal Wistar rats (21 days of age), with an average weight of 40 - 50 grams. Animals were purchased from the Tamil Nadu Veterinary Animal Sciences University (TANUVAS), Chennai. The rats were housed at the SPF animal facility, SRM Institute of Science and Technology, Kattankulathur, Tamil Nadu, India at 23 ± 2°C with 12-hour light/dark cycles for a week to allow them to acclimatize. They were kept in pathogen-free polypropylene cages with corn cob bedding. Each cage contains disposable cardboard tubes for hiding which prevents the aggressive behavior of the animals. The animals were fed a standard diet and free access to double-distilled water. The study was approved by the Institutional Animal Ethical Committee of SRMIST, Chennai, under the CPCSEA guidelines, bearing the number (SAF/IAEC/280622/020).

Experimental animals were divided into five groups:
Group I: Control group (n - 6), Animals were fed a standard diet.
Group II: Animals received LA orally (12 mg/day per Kg body weight) (n-6)
Group III: LA induced rats treated with the broccoli extract orally (300mg/day per Kg BW) (n - 6)
Group IV: LA induced rats treated with vit E orally (50 mg/day per kg BW) (n - 6)
Group V: Animals treated with broccoli extract (n - 6) orally through oral gavage.

For twenty-two days, a similar diet was followed by each group. Following a twenty-two-day trial, the rats were given an overdose of ketamine to induce general anesthesia. To assess biochemical parameters, an intracardiac puncture was used to obtain blood samples. The testis was surgically removed from the rats immediately after they were killed and preserved in 10% formalin to prepare histopathological slides.

Collection of plant: Broccoli was purchased from the local supermarket in Guduvanchery, Chengalpet district, Tamil Nadu, India. The plant was identified and authenticated as *Brassica cretica Lam. syn. Brassica oleracea var. italica Plenck* (Family: Brassicaceae) from Siddha Central Research Institute, Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India by Dr. KN Sunil Kumar, Research officer and HOD, Dept of Pharmacognosy and Dr. P. Elankani, Research officer (Siddha), Sci III/In charge (Form No. PCOG002-ACF.Code: B29032301C, Part: Inflorescence).

Preparation of Broccoli extract: After authentication, fresh broccoli florets were thoroughly rinsed with distilled water to eliminate debris, subsequently, the floret was sliced into small fragments using a sterile blade and left to dry in a shaded area for a duration exceeding two weeks. The resulting sample was coarsely ground using a motor and pestle and then stored in an airtight container for future utilization. Brassica oleracea, the plant from which the sample was derived, was extracted through the utilization of hexane, ethyl acetate, water and ethanol, with an increase in solvent polarity achieved via the cold maceration technique. For every 20 grams of the Brassica oleracea sample, 200 milliliters of solvents were added to a screw-capped bottle, maintaining a ratio of 1:10.

The sample was incubated for 48 hours with continuous stirring and subsequently filtered using a Whatmann no. 1 filter paper. The ethanolic extract was selected for this particular study based on its levels of antioxidants and phytochemical contents. The solvent was then evaporated

using the rotary evaporation method, resulting in the collection of crude extract, which was subsequently employed for further analysis.

Wet weight of testis: The testis was dissected out, free from adherent tissues and washed with normal saline and then the weight was recorded using an electrobalance.

Preparation of tissue homogenate: The weighed testis tissues were homogenized in 0.1 M Tris-HCl buffer (4°C, pH 7.4) using a teflon pestle operated for three minutes at 600 rpm in a Potter-Elvehjem homogenizer. Then, using a Remi refrigerated centrifuge, it was centrifuged for 10 minutes at 3000 xg at 4°C to analyze antioxidant activity.

Estimation of Glutathione (GSH): A reduced glutathione assay was conducted using the Moron et al[21] procedure. To precipitate proteins, 125 µl of 25% trichloroacetic acid (TCA) was added to 0.5 ml of tissue homogenate. The mixture was further diluted with 0.6 milliliters of 5% TCA and centrifuged at 9000 xg for 10 minutes after the tubes were chilled in ice for five minutes. Using a newly prepared DTNB solution (Ellman's Reagent or 5,5-dithiol-bis-(2-nitrobenzoic acid) (2.0 ml) and 0.2 M sodium phosphate buffer (pH 8.0), 0.3 ml of the aliquot was made up to 1 ml.

Estimation of glutathione peroxidase (GPx): The method of Rotruck et al[27] with some changes, was used to assess glutathione peroxidase activity. The reaction mixture containing 0.4 ml buffer, 0.1 ml sodium azide (0.1 ml) and reduced glutathione (0.2 ml), the required amount of enzyme, 0.1 ml hydrogen peroxide and water was taken to a final incubation volume 2.0 ml. The tubes were kept for 10 mins. at 30°C. The reaction was arrested by adding TCA (0.5 ml). For finding the residual GSH content, the supernatant was taken and added to the precipitating reagent (2.0 ml) and DTNB reagent (1.0 ml). At 412 nm, the color was read. A blank was prepared with only sodium dihydrogen phosphate and DTNB reagent (1.0 ml). Suitable aliquots of the standard were taken and treated in a similar way. The activity was measured in moles of GSH utilized/sec/mg protein.

Estimation of lipid peroxide (LPO) or Malondialdehyde (MDA): According to Okhawa et al[24], TBARs were used to measure the amount of lipid peroxide in tissues. To 0.5 ml of tissue homogenate, 20% acetic acid (1.5 ml), SDS (0.2 ml) and TBA (1.5 ml) were added. Using distilled water, the mixture was made up to 4.0 ml and then it was heated at 95°C (60 minutes) using a glass ball as a condenser. After cooling, butanol-pyridine mixture (4.0 ml) was added and shaken well. After centrifugation (10 min) at 4,000 rpm, absorbance was read at 532 nm using the organic layer. Standard and blank were treated in a parallel manner. The lipid peroxide concentration was measured in moles of MDA formed/g tissue.

Estimation of Superoxide dismutase (SOD): SOD activity was examined in accordance with Misra and Fridovich's[19] methodology. In the tubes containing 0.5ml of carbonate buffer and EDTA solution (0.5 ml), the required amount of enzyme was added and the final volume was made up to 2.5ml. The reaction began by adding epinephrine (0.4 ml). The increased absorbance was read (480 nm) by a Shimadzu UV spectrophotometer. Epinephrine to adrenochrome 50% autoxidation was executed in a control tube without the enzyme. The enzyme activity was measured in units/mg protein.

Estimation of Catalase (CAT): Catalase activity was assessed using Sinha's[31] procedure. 0.05 ml of tissue homogenate was added to 1.2 ml of the phosphate buffer. To this, 1.0 ml of hydrogen peroxide was added to start the enzyme reaction. The decreased absorbance was taken (620 nm) at 30-sec intervals for 3 minutes. The enzyme blank was run using distilled water (1ml). Activity of catalase was measured in moles of H_2O_2 decomposed /min/mg protein.

Estimation of the levels of gonadotropin and testosterone hormones: Serum levels of FSH, LH and testosterone were assessed by using an Enzyme-linked immunosorbent assay. All the assays were performed using commercial Elisa kits. The kits were obtained from Cusa Bio, Houston, TX 77054, USA. The assay was performed according to the manufacturer's guidelines.

Sperm Analysis

Sperm morphology: To analyze the morphology of sperm, one drop of approximately 20 µL of the sperm suspension was put onto the microscope slides and swiped. The slides were then dried and stained with eosin-nigrosin (eosin Y 1% and nigrosine 5%). It was then examined at 400 x magnifications in an optical microscope (Olympus Optical, Japan). A count of 200 spermatozoa per slide was carried out and changes were noted for the head, middle piece and tail. Results were stated as the percentage of abnormal cells.

Sperm motility: The epididymis of rats was excised after sacrifice. The Olympus Research Microscope (Olympus, Japan) was used to evaluate the sperm profile under a microscope. Under 400x magnification, a diluted sperm suspension was made and forward progressing motility was examined on a pre-warmed slide. The motility was then scored to the nearest 10[25,33].

Sperm viability: An equal volume of nigrosine-eosin (1% eosin Y and 5% nigrosine) (SD Fine Chemicals Ltd, Chennai) was added to a sample of 20 µL of the sperm-containing solution. Following content smearing on the microscope slide and drying, the prepared materials were examined at 400x magnification using light microscopy.

By comparing the percentage of sperm that were unstained (complete membrane, considered viable) to those that were colored (impaired membrane, considered non-viable), an individual count of 200 spermatozoa was carried out. The

percentage of spermatozoa with intact membranes (viable) among all sperm counted was used to express the results[7,25].

Sperm count: The sperm count was estimated using a Neubauer hemocytometer. Under a light microscope, the number of motile spermatozoa in each of the grid's ten squares was counted and the mean number of sperm was noted. The formula (mean number of motile spermatozoa/total number of spermatozoa) × 100% was used to calculate the percentage of motile spermatozoa.

Histopathological study of testis: To prepare for histological slides, the tissues of the right testis were preserved in 10% buffered formalin for 24 hours. After being dehydrated, testicular tissues were washed in xylene and embedded in paraffin wax. Paraffin slices that were 4-6 µm thick, were made with LKB ultramicrotome and stained with hematoxylin and eosin (H&E).

Ki67 – Immunohistochemistry of testis: The testes were stained with a Ki67 special stain for the assessment of proliferative cells. The method mentioned by Joon et al[14] was used to assess the positive Ki67 cells in the seminiferous tubules. Under 100x magnification, cells were counted at random (5 seminiferous tubules per testis) and the mean value was subsequently calculated. A cell-proliferated antigen called Ki67 was utilized to identify germ cells going through mitosis.

Morphometry of seminiferous tubules: Using the method outlined by Subramanian et al[32], the diameter of the seminiferous tubules, the luminal diameter and the epithelial thickness were measured. Testicular parameters were randomly measured at 10x magnification in 10–12 transverse sections of seminiferous tubules (10 seminiferous tubules for each slide).

Comet assay/single cell gel electrophoresis (SCGE) assay: After obtaining a sperm cell, the cells were immersed in tissue homogenate (50 µL) mixed with 200 µL of 0.5% low liquefaction point agarose and positioned on a slide that had been previously coated with a layer of routine agarose at a concentration of 0.75%. To level the agarose layer, a coverslip was used and then refrigerated for 10 minutes to allow the gel to dry. The coverslip was delicately lifted, followed by the addition of a new layer of low liquefaction point agarose and the process was reiterated. Once the agarose gel had hardened, the slides were immersed in a lysis solution at pH 10 (2.5 M NaCl, 100 mM Na_2 EDTA, 10 mM Tris pH 10, 1% Triton X-100, 10% DMSO) containing high salts and detergents for the final hour.

At the terminal stage of the lysing duration, the slides underwent incubation in an alkaline buffer solution (with a pH lower than 13.0) for 25 mins, before proceeding to electrophoresis. This incubation step aimed to generate single-stranded DNA and to induce the formation of alkali labile sites (ALS) as single-strand breaks (SSB). The alkaline solution was made up of 1 millimolar EDTA and 300 millimolar NaOH. During the process of electrophoresis, the alkaline buffer employed was identical to the pH less than 13 buffer utilized for alkali uncoiling. The electrophoretic setting utilized was 25 Voltage and 300 mA, then the DNA was subjected to electrophoresis for a duration of 20 minutes under a dark environment and at a temperature of 58°C[33]. This lower temperature was chosen since it is believed to enhance the reproducibility of the results.

Following electrophoresis, the gels were made neutral by immersing the slides in 0.4 M (pH 7.5) tris solution three times, with each rinse lasting for 5 minutes[22]. The slides were subsequently stained with 20 µg/mL EtBr and the comets (damaged DNA tail length) were evaluated using an inverted fluorescence microscope (Olympus IX70) equipped with a 40x objective and a 340 nm exciter filter. Images were captured with a digital camera with networking capability and were analyzed by image analysis software (Version 2).

Statistical Analysis: Data were analyzed using one-way Analysis of Variance (ANOVA) followed by post-hoc multiple comparison tests to determine significant differences among the experimental groups. Results were expressed as mean ± standard deviation (SD). The significance level was set at $p < 0.05$. Statistical analyses were performed using SPSS software.

Results

Comparison of Testis Weight (g/100g Birth Weight) among Groups of Prepubertal Male Wistar Rats: The analysis of testis weight (g/100g birth weight) among the groups showed statistically significant differences ($p = 0.001$) represented in figure 1. The control group exhibited the highest mean testis weight (0.72 ± 0.01), indicating optimal reproductive organ development. In contrast, the lead-exposed group had the lowest mean value (0.64 ± 0.01), highlighting the detrimental effects of lead toxicity. The groups treated with ethanolic extract of broccoli (Broc) and vitamin E (LA + Vit E) showed moderate recovery with mean values of 0.69 ± 0.01 and 0.67 ± 0.01 respectively. The combination treatment of lead and broccoli extract (LA + Broc) resulted in a slightly lower mean (0.66 ± 0.01). These findings suggest that broccoli extract and vitamin E have ameliorative effects against lead-induced reproductive toxicity, with broccoli extract showing marginally higher efficacy.

Effects of Broccoli Extract on LPO and Antioxidant enzymes in testis of Lead toxicity-induced Pre-Pubertal Rats: Effects of broccoli extract on LPO and antioxidant enzymes in testis of lead toxicity-induced Pre-Pubertal Rats are represented in table 1. LPO levels were significantly reduced in the broc and control groups compared to the lead group, underscoring the protective role of broccoli extract in mitigating oxidative stress. Additionally, all antioxidant parameters showed statistically significant differences among the groups ($p = 0.001$ for all). The broc group

recorded the highest levels of GPX, SOD and GSH, highlighting its superior antioxidant activity whereas the lead group displayed the lowest levels, reflecting severe oxidative damage. These findings suggest that broccoli extract effectively boosts antioxidant defenses in comparison to lead exposure.

Effects of Broccoli Extract on reproductive hormones in Lead toxicity-induced Pre-Pubertal Rats: The levels of reproductive hormones including testosterone, FSH and LH, differed significantly across the groups (p = 0.001) presented in figure 2. The Broc group exhibited the highest mean testosterone (2.9 ng/ml), FSH (4 mIU/ml) and near-highest LH (3.78 mIU/ml), demonstrating its potent protective effect against lead-induced reproductive toxicity. The control group showed slightly lower levels of testosterone (2.77 ng/ml) and FSH (3.95 mIU/ml) but the highest LH levels (3.93 mIU/ml). Intermediate levels were observed in the LA + Vit E and LA + Broc groups, with testosterone ranging from 1.71–1.89 ng/ml, FSH from 2.75–2.83 mIU/ml and LH from 2.26–2.34 mIU/ml.

The lead group had the lowest values for all hormones, with testosterone at 0.92 ng/ml, FSH at 2.51 mIU/ml and LH at 1.9 mIU/ml, highlighting severe suppression of reproductive function. These results demonstrate that broccoli extract significantly mitigates lead-induced hormonal disruptions, underscoring its potential as a protective agent.

Effects of Broccoli Extract on Sperm Parameters of Lead toxicity-induced Pre-Pubertal Rats: The differences in sperm motility, viability, count and abnormal morphology were statistically significant across all groups (p = 0.001 for all) (Table 2). The Broc group demonstrated the highest levels of sperm motility, viability and count, while the lead group exhibited the lowest. Conversely, sperm abnormal morphology was most prominent in the lead group and least in the control group, indicating significant protective effects of broccoli extract against lead-induced reproductive toxicity.

Effects of ethanolic extract of Broccoli on histopathology of testis of experimental rats: Figure 3 shows the histopathology of the testis of experimental rats. In the control group, numerous seminiferous tubules lined by stratified epithelium show the following cells: spermatogonia, spermatocytes, spermatids and the tail of spermatozoa in the lumen of tubules. In interstitial connective tissue, the Leydig cells were observed. In the lead acetate group, the appearance of intertubular edema of the testis, congestion of blood vessels, sloughing of spermatogonia and accumulation of the sloughed cells were detected in the centre of the seminiferous tubules, karyolysis of spermatocytes, fewer spermatozoa in the lumen, disintegration and degeneration of cells of seminiferous tubules including Sertoli cells and Leydig cells (scanty), thickened and detached basement membrane. In the LA+BR treated group, the seminiferous tubules showed less congestion and slight edema, Sertoli cells and spermatocytes appear normal, with an increased number of spermatozoa in the lumen of tubules, reduced regeneration of cells of seminiferous tubules and adequate Leydig cells. The LA+Vit E treated group showed reduced testicular injury. In the Broccoli group, normal seminiferous tubules (spermatogonia, spermatocytes, spermatids, spermatozoa, interstitial connective tissue and Leydig cells) were observed without any pathological damage.

Effects of ethanolic extract of Broccoli on Ki67-Positive Cells (%) expression in Lead toxicity-induced Pre-Pubertal Rats: The percentage of Ki67-positive cells showed a statistically significant difference across all the groups (p = 0.001) as represented in figure 4. The broc group exhibited the highest mean percentage of Ki67-positive cells (73.27%), indicating enhanced cellular proliferation, followed by the control group (66.35%), LA + Vit E group (50.92%) and LA + Broc group (45.95%). The lead group had the lowest percentage of Ki67-positive cells (23.62%), reflecting a marked reduction in cellular proliferation, likely due to lead-induced toxicity. These results suggest that broccoli extract significantly promotes cell proliferation compared to the lead-exposed group.

Effects of ethanolic extract of Seminiferous Tubule Diameter, Luminal Diameter and Epithelial Thickness in Lead toxicity-induced Pre-Pubertal Rats: The comparative analysis of seminiferous tubule diameter, luminal diameter and epithelial thickness among the groups of pre-pubertal male Wistar rats revealed significant differences ($p< 0.05$) (Table 3). The seminiferous tubule diameter was highest in the broccoli (Broc) group (27.25 ± 5.00 µm), followed by the control group (21.38 ± 0.76 µm), LA + broc group (19.48 ± 0.42 µm), LA + Vit E group (18.06 ± 0.43 µm) and the lead acetate (Lead) group (16.19 ± 0.94 µm). Similarly, the luminal diameter was significantly greater in the broc group (8.06 ± 1.08 µm) compared to the control (6.76 ± 0.31 µm), LA + Broc (5.78 ± 0.25 µm), LA + Vit E (5.17 ± 0.24 µm) and lead group (4.44 ± 0.37 µm).

The epithelial thickness followed a comparable trend with the broc group showing the highest value (75.29 ± 9.78 µm), followed by the control (55.75 ± 8.79 µm), LA + broc (31.13 ± 5.84 µm), LA + Vit E (19.05 ± 5.04 µm) and lead group (14.29 ± 4.18 µm). The Broc group exhibited significant improvement across all parameters compared to the lead group ($p< 0.05$) while the LA + Vit E and LA + Broc groups showed partial amelioration, with the latter demonstrating superior recovery.

Effects of ethanolic extract of Broccoli on DNA Tail Length in Lead toxicity-induced Pre-Pubertal Rats: DNA tail length, a marker of DNA damage, also differed significantly among the groups (p = 0.001), as shown in figure 5. The lead group had the highest mean tail length (208.5), indicating severe DNA damage, followed by the LA + broc group (160.5), LA + Vit E group (88.17) and the

control group (51). The broc group showed the lowest mean DNA tail length (45), demonstrating its protective effect in mitigating DNA damage caused by lead exposure. These findings highlight the significant role of broccoli extract in reducing oxidative DNA damage and maintaining genomic integrity.

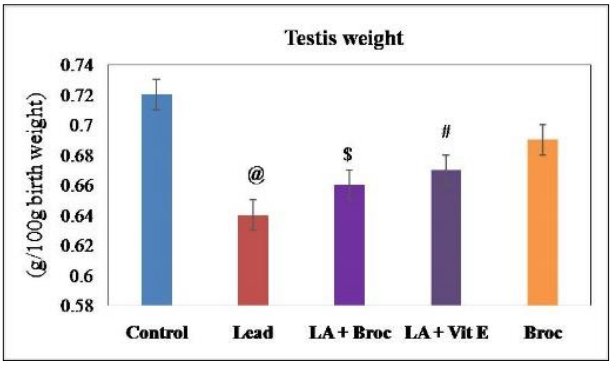

Figure 1: Effect of ethanolic extract of Broccoli on Testis Weight (g/100g Birth Weight) in Lead toxicity induced Pre-pubertal Male Wistar Rats. Each bar represents Mean ± SEM of 3 observations representing 6 animals. Significance at p<0.01. @- compared with control, $- compared with Lead toxicity induced test group, #- Compared with lead toxicity induced group treated with broccoli.

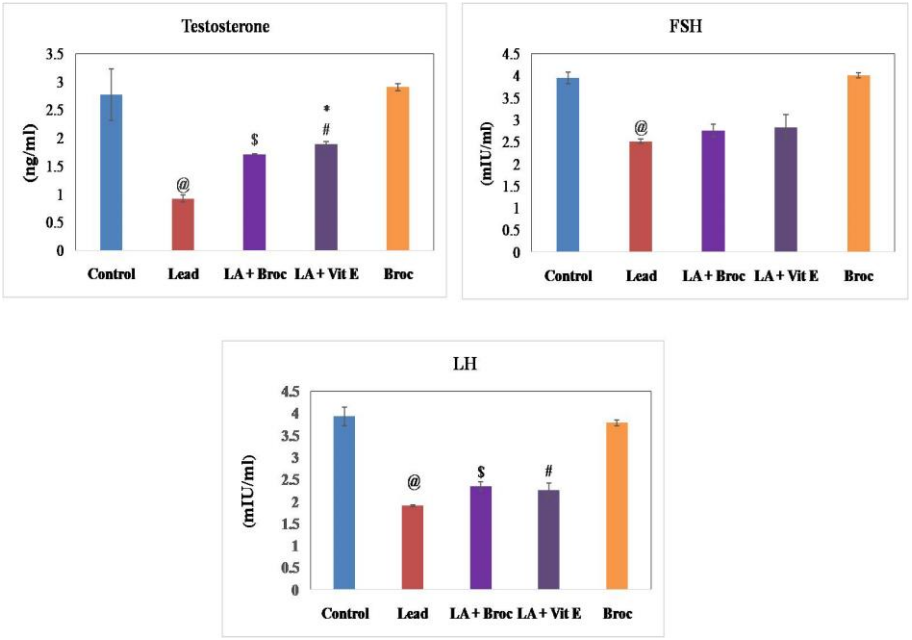

Figure 2: Effect of ethanolic extract of Broccoli on Reproductive Hormones in Lead toxicity induced Pre-pubertal Male Wistar Rats. Each bar represents Mean ± SEM of 3 observations representing 6 animals. Significance at p<0.01. @- Compared with control, $- Compared with Lead toxicity induced test group, #- Compared with lead toxicity induced group treated with broccoli, *- Compared with control treated with Broccoli.

Table 1
Comparison of oxidative stress and Antioxidant Parameters among experimental Groups of Prepubertal Male Wistar Rats

Group	LPO (nM/mg tissue)	SOD (mU/mg tissue)	CAT (µM/mg tissue)	GSH (nM/mg tissue)	GPX (mU/mg tissue)
Control	0.55 ± 0.03	70.02 ± 0.60	43.98 ± 1.65	3.26 ± 0.04	2.65 ± 0.03
Lead Acetate treated Group	0.85 ± 0.02$^{@}$	44.40 ± 1.35$^{@}$	28.82 ± 0.54$^{@}$	0.89 ± 0.03$^{@}$	1.05 ± 0.03$^{@}$
LA + Broc	0.64 ± 0.03$^{\$}$	51.30 ± 0.52$^{\$}$	37.08 ± 0.35$^{\$}$	2.92 ± 0.04$^{\$}$	2.19 ± 0.01$^{\$}$
LA + Vit E	0.64 ± 0.03*	53.98 ± 0.74*	36.45 ± 3.45*	3.11 ± 0.09*	2.41 ± 0.17*
Control + Broc	0.56 ± 0.03$^{\#}$	72.90 ± 1.45$^{\#}$	43.97 ± 0.69$^{\#}$	3.36 ± 0.02$^{\#}$	2.78 ± 0.04$^{\#}$

@: Significant difference between Lead vs. Control ($p < 0.001$); $: Significant difference between Lead vs LA + Broc ($p < 0.001$); *: Significant difference between LA + Broc vs LA + Vit E ($p < 0.001$); #: Significant difference between Broc vs Control ($p < 0.001$)

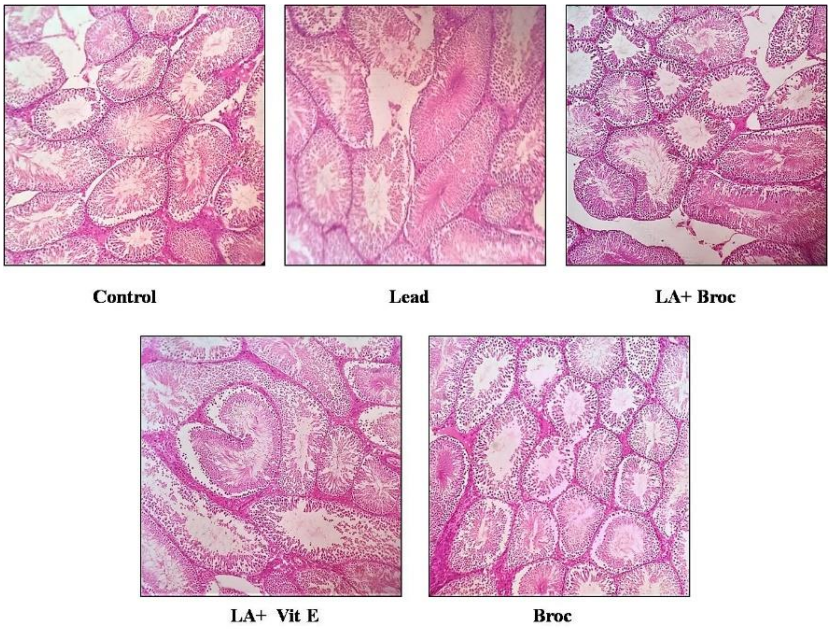

Figure 3: Histology of Testis Tissue in Lead toxicity induced Pre-pubertal Male Wistar Rats. Histological changes in testis across experimental groups: (A) Control group showing normal seminiferous tubules with stratified epithelium and typical spermatogenic cells. (B) Lead acetate group displaying intertubular edema, congestion, sloughing of spermatogonia, karyolysis of spermatocyte, reduced spermatozoa and degenerated Sertoli and Leydig cells. (C) LA+BR treated group exhibiting reduced congestion and edema, normal Sertoli cells, increased spermatozoa and adequate Leydig cells. (D) LA+Vit E treated group showing mitigated testicular injury. (E) Broccoli group showing normal seminiferous tubules and interstitial tissue without pathological changes.

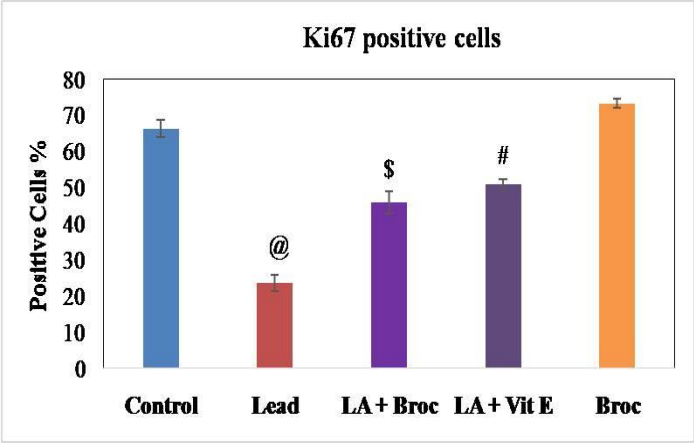

Figure 4: Effect of ethanolic extract of Broccoli on Ki67 positive cells in Lead toxicity induced Pre-pubertal Male Wistar Rats. Each bar represents Mean ± SEM of 3 observations representing 6 animals. Significance at p<0.01. @- Compared with control, $- Compared with Lead toxicity induced test group, #- Compared with lead toxicity induced group treated with broccoli, *- Compared with control treated with Broccoli.

Table 2
Comparisons of Sperm Parameters among Groups of Prepubertal Male Wistar Rats

Group	Sperm Motility (%)	Sperm Viability (%)	Sperm Count (million/ml)	Sperm Abnormal Morphology (%)
Control	61.67 ± 0.82	71.53 ± 0.81	20.92 ± 1.14	1.22 ± 0.12
Lead	41.33 ± 1.21$^{@}$	54.83 ± 0.56$^{@}$	12.90 ± 0.24$^{@}$	5.23 ± 0.41$^{@}$
LA + Broc	50.00 ± 1.41$^{\$}$	60.45 ± 0.83$^{\$}$	15.80 ± 0.45$^{\$}$	4.32 ± 0.19$^{\$}$
LA + Vit E	53.50 ± 1.87*	64.25 ± 0.50*	17.95 ± 0.72*	3.45 ± 0.33*
Broc	60.17 ± 3.66$^{\#}$	70.43 ± 0.96$^{\#}$	22.10 ± 1.75$^{\#}$	1.48 ± 0.17$^{\#}$

@: Significant difference between Lead vs Control ($p < 0.001$); $: Significant difference between Lead vs LA + Broc ($p < 0.001$); *: Significant difference between LA + Broc vs LA + Vit E ($p < 0.001$) #: Significant difference between Broc vs Control ($p < 0.001$).

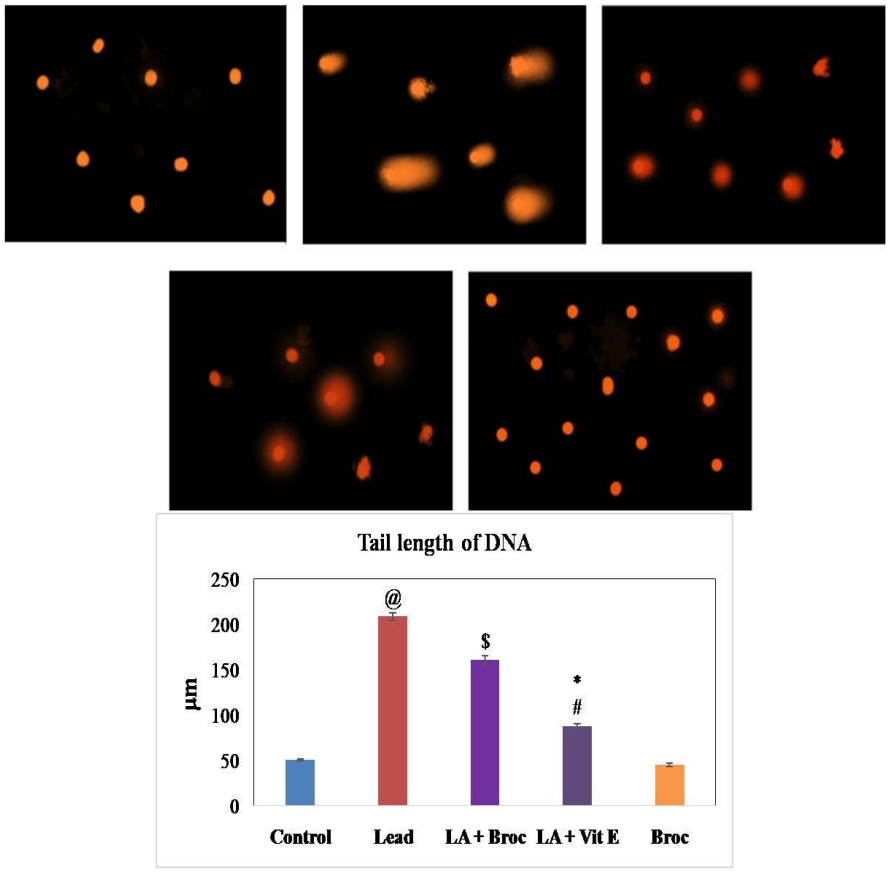

Figure 5: Effect of ethanolic extract of Broccoli on DNA tail length in Lead toxicity induced Pre-pubertal Male Wistar Rats. Each bar represents Mean ± SEM of 3 observations representing 6 animals. Significance at p<0.01. @- Compared with control, $- Compared with Lead toxicity induced test group, #- Compared with lead toxicity induced group treated with broccoli, *- Compared with control treated with Broccoli

Table 3
Comparisons of Seminiferous Tubule Diameter, Luminal Diameter and Epithelial Thickness among Groups of Prepubertal Male Wistar Rats

Group	Seminiferous Tubule Diameter (µm)	Luminal Diameter (µm)	Epithelial Thickness (µm)
Control	21.38 ± 0.76	6.76 ± 0.31	55.75 ± 8.79
Lead Acetate	16.19 ± 0.94$^{@}$	4.44 ± 0.37$^{@}$	14.29 ± 4.18$^{@}$
LA + Broc	19.48 ± 0.42$^{\$}$	5.78 ± 0.25$^{\$}$	31.13 ± 5.84$^{\$}$
LA + Vit E	18.06 ± 0.43*	5.17 ± 0.24*	19.05 ± 5.04*
Control+ Broc	27.25 ± 5.00$^{\#}$	8.06 ± 1.08$^{\#}$	75.29 ± 9.78$^{\#}$

@: Significantly different compared to the Control group ($p< 0.05$).
$: Significant amelioration compared to the Lead Acetate group ($p< 0.05$).
#: No significant difference compared to the Control group ($p> 0.05$).
*: Significant amelioration compared to the Lead Acetate group but lower than Broc alone ($p< 0.05$).

Discussion

The present study highlights the protective effects of broccoli extract on reproductive and biochemical parameters in pre-pubertal male Wistar rats exposed to lead toxicity. The statistically significant reduction in testis weight observed in the lead-exposed group is consistent with findings from Flora et al who reported impaired testicular development due to oxidative stress and disruption of endocrine function by lead exposure. Lead-induced reduction in testis weight may result from inhibited spermatogenesis and Leydig cell damage, as previously described by Iavicoli et al[12].

The partial recovery in testis weight observed in the broccoli and vitamin E-treated groups emphasizes their antioxidant and protective properties in counteracting lead toxicity, with broccoli extract showing marginally better efficacy, likely due to its sulforaphane content. The oxidative stress induced by lead exposure, evidenced by elevated lipid peroxidation (LPO) levels and reduced antioxidant enzyme activity, corroborates prior studies linking lead toxicity to excessive free radical production[23]. Broccoli extract significantly reduced LPO levels and restored antioxidant enzyme activity, including GPX, SOD and GSH. These findings are

consistent with studies by Mohammadi et al who demonstrated the potent free radical-scavenging properties of sulforaphane and other bioactive compounds in broccoli[26].

Enhanced antioxidant enzyme levels in the broccoli-treated group suggest its superior efficacy in mitigating oxidative stress compared to vitamin E. Reproductive hormone analysis revealed significant suppression of testosterone, FSH and LH in the lead group, reflecting endocrine disruption caused by lead toxicity. Lead has been shown to disrupt the hypothalamic-pituitary-gonadal axis and damage Leydig cells, resulting in hormonal imbalance[17]. The broccoli extract-treated group demonstrated near-normal levels of testosterone and FSH, with the highest LH levels observed in the control group. These improvements align with Wang et al[37] who reported the ameliorative effects of dietary antioxidants on hormone regulation. Broccoli's sulforaphane may play a role in reducing inflammation and protecting gonadal tissues, thereby restoring hormonal homeostasis[36].

Sperm parameter analysis revealed significant differences in motility, viability, count and morphology among groups. The marked decline in sperm quality in the lead-exposed group is consistent with studies showing that lead-induced oxidative stress disrupts spermatogenesis and damages sperm DNA[15]. The broccoli extract-treated group exhibited the highest sperm motility and count and the lowest abnormal morphology, indicating its potential to protect and enhance spermatogenic function. The improvements in sperm quality may result from the reduction of oxidative damage and preservation of testicular architecture.

Cellular proliferation, as indicated by Ki67-positive cells, was significantly enhanced in the broccoli group compared to lead-exposed rats. Lead exposure suppresses cell proliferation in the testes, potentially impairing spermatogenesis and testicular growth[4]. Broccoli extracts significantly improved Ki67-positive cell percentages, suggesting its potential role in testicular tissue regeneration. This finding supports prior studies emphasizing the role of dietary antioxidants in promoting cellular proliferation and recovery[36].

The observed reductions in seminiferous tubule diameter, luminal diameter and epithelial thickness in the lead acetate group align with previous studies indicating that lead exposure adversely affects testicular histomorphometry. Lead-induced oxidative stress disrupts spermatogenesis, leading to structural degeneration of seminiferous tubules. For instance, a study by Li et al[18] reported significant decreases in seminiferous tubular diameter and epithelial height following exposure to lead acetate. Such structural alterations are attributed to oxidative stress and apoptosis in testicular cells[3]. Similar findings were reported by Zhang et al[39] who demonstrated that lead exposure causes histological degeneration and functional impairment in the testes[2].

The administration of broccoli extract (Broc) demonstrated a protective effect, as evidenced by the significant improvements in seminiferous tubule diameter, luminal diameter and epithelial thickness compared to the lead Acetate group. Broccoli is rich in antioxidants such as sulforaphane, which can mitigate oxidative stress and promote tissue repair. A study by Abdalla et al[1] evaluating the antioxidative effects of broccoli extract found significant improvements in testicular histology and spermatogenic function in rats exposed to oxidative stress. Additionally, the anti-inflammatory properties of broccoli have been documented, further supporting its role in ameliorating testicular damage[5].

The partial amelioration observed in the LA + Broc and LA + Vit E groups indicates that combining lead exposure with antioxidant treatments can confer some degree of protection against testicular damage. Vitamin E, a well-known antioxidant, has been shown to exert protective effects against testicular toxicity induced by various agents. For example, co-administration of vitamin E and selenium mitigated lead-induced testicular damage in prepubertal rats, as evidenced by improvements in seminiferous tubule structure[16]. Similarly, curcumin has demonstrated protective effects against lead acetate-induced testicular toxicity by enhancing antioxidant enzyme activities and reducing oxidative stress[30].

The deleterious effects of lead on testicular histomorphometry are well-documented, with significant reductions in seminiferous tubule diameter, luminal diameter and epithelial thickness. Broccoli extract exhibits a protective role, likely due to its antioxidant and anti-inflammatory properties, leading to improvements in these parameters. The partial amelioration observed with combined treatments suggests that antioxidant therapy may offer a viable strategy to mitigate lead-induced testicular damage.

The analysis of DNA damage through tail length measurements further underscores the protective role of broccoli extract. Lead-induced DNA damage, as indicated by increased tail length in the lead group, reflects genotoxicity resulting from oxidative stress[23]. Broccoli extracts significantly reduced tail length, highlighting its protective effects in maintaining genomic stability. This result aligns with Fahey et al[9] who emphasized sulforaphane's ability to induce phase II detoxifying enzymes, thereby protecting against DNA damage[6].

Our recent study further supports the broader protective effects of broccoli extract in lead-induced toxicity. In this study, we demonstrated the renoprotective effects of broccoli extract in pre-pubertal Wistar rats exposed to lead acetate toxicity. We observed that the administration of broccoli extract resulted in significant improvements in kidney function, as evidenced by reduced levels of serum creatinine, urea and other markers of renal damage.

Similar to its effects on the reproductive system, broccoli extract in our earlier study was shown to mitigate oxidative stress and prevent histopathological damage in the kidneys, likely through its potent antioxidant properties. These findings align with the current results, where broccoli extract also demonstrated remarkable improvements in antioxidant enzyme activities (GPX, SOD and GSH) and reduced oxidative DNA damage in the testis, further supporting the extract's role as a comprehensive protective agent against lead-induced toxicity. The renoprotective and reproductive benefits of broccoli extract, when used as a dietary supplement, underscore its potential as a natural therapeutic for mitigating heavy metal toxicity in various organ systems[24].

Conclusion

The findings of this study underscore the significant protective effects of broccoli extract against lead-induced toxicity in pre-pubertal Wistar rats. Broccoli extract demonstrated its potential to mitigate oxidative stress by reducing lipid peroxidation and enhancing antioxidant enzyme activity, such as GPX, SOD, LPO, CAT and GSH. Furthermore, it effectively restored reproductive hormone levels, sperm parameters and testicular weight, highlighting its role in preserving reproductive health.

Broccoli extract also promoted cellular proliferation, increased Ki67-positive cells and reduced DNA damage, as reflected by shorter DNA tail lengths. Collectively, these results suggest that broccoli extract serves as a potent natural intervention against lead-induced reproductive and oxidative damage, offering a promising avenue for therapeutic applications. Future studies are warranted to explore its molecular mechanisms and potential clinical translation.

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