

Protective Effect of Selenium and Zinc Against Dehp Induced Toxicity in the Thyroid Gland of Rats

Nabiha Hussain, Shabana Ali, Maleeha Bano*, Tooba Khurshheed, Huma Beenish, Syeda Sara Bano**

Islamic International Medical college, Islamabad Pakistan, *HITEC Institute of Medical Sciences, Taxila/National University of Medical Sciences (NUMS) Pakistan, **Shifa college of Medicine, Islamabad Pakistan

ABSTRACT

Objective: To compare the efficacy of Selenium and Zinc in improving histological features on DEHP-induced toxicity in the thyroid gland of rats.

Design of study: Laboratory based experimental study.

Place and Duration of Study: Anatomy Department of Islamic International Medical College with the National Institute of Health (NIH), Islamabad Pakistan cooperation from Sep 2019 to Sep 2020.

Methodology: Forty, eight weeks old rats were divided into four groups, each containing ten male rats. In control, Group-A rats were on the standard diet. In Group-B, rats were given orally 500mg/kg/day DEHP. In Group-C rats were given 500mg/kg/day DEHP and 1mg/kg/day Selenium orally. In Group-D rats were given 500mg/kg/day DEHP and 5mg/kg/day Zinc orally. After 28 days, rats were dissected, and the thyroid gland was removed. Histological parameters, vacuolization and number of follicular cells were observed with H and E staining among control and experimental groups.

Results: In Group-B, 50% of rats showed severe vacuolization. In Group-C, 60% of rats showed minimal vacuolization. In Group-D, 40% of rats showed minimally, and 40% of rats showed mild vacuolization. Among quantitative parameters, the number of follicular cells was conserved more by Selenium than Zinc.

Conclusion: Selenium and Zinc have a protective effect on thyroid histological changes caused by DEHP, but Selenium shows better conservation than zinc on thyroid histology.

Keywords: DEHP, Selenium, Oxidative stress, Zinc.

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INTRODUCTION

Phthalates exist universally in the human community. Among phthalates, Di-(2-Ethylhexyl) phthalate DEHP is extensively utilized within the production of polyvinylchloride that is applied in numerous products such as flooring constituents, plastic toys, tubing, hospital devices, also in cosmetics and personal care items. Human beings are exposed to a high degree of phthalates via oral, inhalation and dermal routes.¹ Phthalates are used in PVC materials, including lid gaskets, tubings used in the milking process, food preparation gloves and food wrapping materials.² Through these materials, phthalates leach into food. It is loosely bound to PVC, so it is easily leached out from PVC products to the environment, and high temperatures increase the migration.³ DEHP exposure from packaged food was 2 mg/day in inhabitants.⁴ A study reported that people who used to eat more fast food have 23.8% greater levels of DEHP than non-consumers who did not take any fast food in the last 24 hours.^{5,6} DEHP is converted into mono-ethyl-5-

hydroxyhexyl phthalate (MEHP) by secondary oxidation,⁷ leading to oxidative stress in the reproductive system, liver, kidneys, and thyroid gland. DEHP affects the thyroid gland by producing reactive oxygen species. It also induces hepatic enzymes, contributing to the down-regulation of thyroid hormones.⁸ It causes mutation at the mRNA level, leading to the suppression of the biosynthesis of thyroid hormones.

The thyroid gland maintains an optimal metabolism level in the tissues necessary for their normal function. Normal thyroid functioning depends on the presence of various trace elements, including iodine, Selenium (Se), Zinc (Zn) and iron. Balanced expression of the selenoproteins that act as oxidoreduc-tases and redox signal regulators requires adequate selenium intake.⁹ Among Selenoproteins, glutathione peroxidase has an antioxidant effect, whereas Iodothyronine deiodinases synthesise active thyroid hormone.¹⁰ Selenium has positive effects on improving hypothyroidism. Another trace element is zinc which has antioxidant activity and plays an important role in thyroid hormone metabolism. Zinc increases thyroid hormone synthesis by increasing the level of thyrotropinre-

Correspondence: Dr Nabiha Hussain, House No- 1549. 12th Avenue Service Road G-11/2, Islamabad Pakistan

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leasing hormone. It also helps in the conversion of thyroxine to triiodothyronine T3. The study aimed to compare the efficacy of Selenium and Zinc in improving histological features on DEHP-induced toxicity in the thyroid gland of rats.

METHODOLOGY

It was a laboratory based experimental study, conducted at Anatomy Department of Islamic International Medical College with the National Institute of Health (NIH), Islamabad Pakistan cooperation from September 2019 to September 2020 after the approval Ethical Review Committee, Islamic International Medical College, Rawalpindi.

Inclusion Criteria: Adult male rats of age above two months and weighing 250gm to 300gm were included in the study.

Exclusion Criteria: Female rats and rats weighing <200gm and >300gm were excluded from the study.

This study was conducted at the Animal House of NIH, Chak Shehzad Islamabad. DEHP in liquid form with 99% purity was purchased from the science centre in Rawalpindi. Selenium yeast (organic) and Zinc Gluconate were purchased from GNC store Islamabad.

Forty rats weighing (250-300gm) were kept under standard temperature in an air-conditioned room. 40 Albino adult male rats were used in the study as a mammalian models. They were shifted into clean stainless steel cages under 12-hour light and dark cycle. They were given food and water ad libitum for 7-days to acclimatize. Rat pellets and water were used as food during the whole experiment. Each group was comprised of 10 male rats. All groups were treated for 28 days.

Group-A was the control group given a standard diet and tap water. Group-B was given a standard diet and 1.5 ml of DEHP orally daily with food for four weeks. Group-C was given a standard diet and 1.5 ml of DEHP with food and Se at a dose of 3mg dissolved in drinking water. Group-D was given a standard diet and 1.5 ml of DEHP with food and Zinc at a dose of 15mg in drinking water.

DEHP was given orally along with diet at a dose of 500mg/kg/rat for experimental Groups B, C, and D. For each group, 1.5 ml of DEHP was measured by a 5cc syringe and given along with the food. Selenium yeast (organic) and Zinc Gluconate were given according to the dose of 1mg/kg/day and 5mg/kg/day in powdered form. The powdered form was weighed on an electronic scale and then dissolved in tap water to

make a 20ml solution for each rat which was given daily to the experimental group via the oral route.

After 28 days of the experiment, rats were euthanized and dissected. Thyroid glands were fixed in 10% of formalin, stained with Hematoxylin and Eosin. The magnification of power10X of a light micro-scope was used to examine slides. Microscopic qualitative parameter vacuolization was classified according to the International Harmonization of Toxicologic Pathology Nomenclature. The number of follicular cells was counted in five follicles for each thyroid section per animal in H&E stained slides (10×10X). The count tool of Image J was used to count the number of follicular cells. Counting was started from one point of the follicle to include all cells in that follicle by coming back to the initial point in the clockwise direction in each follicle.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were summarized as Mean±SD and qualitative variables were summarized as frequency and percentages. One-way analysis of variance (ANOVA) was applied to find out the mean differences among the groups. Post Hoc Tukey's test was applied for further inter-group comparisons. The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

Group-A showed negligible vacuolization in 9 rats. In Group B, 50% of rats showed severe, and 30% of rats showed moderate vacuolization. In Group C, 60% of rats showed minimal, and 20% of rats showed negligible vacuolization. Finally, in Group-D, 40% of rats showed minimal, and 40% of rats showed mild vacuolization (Figure).

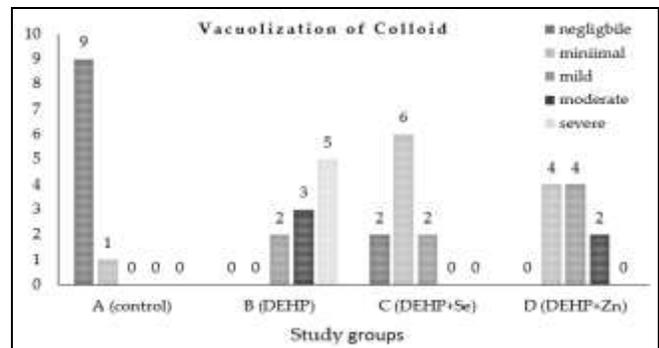


Figure: Grades of Vacuolization among Control and Experimental groups(n=40)

Regarding quantitative parameters, the mean number of follicular cells in Group-A was 17.36±2.31.

The mean number of follicular cells in Group-B was 45.62 ± 1.9 , significantly more than in Group-A (Table-I).

Table-I : Group wise Distribution of Total Number of cells in follicles among Control and Experimental Groups of Albino rats (n=40)

Total Number of Cells In A Follicle (In Counts)					p-value
Groups	A (Control)	B (DEHP)	C (DEHP +Se)	D (DEHP +Zn)	
Mean± SD	17.36± 2.31	45.62± 1.95	18.94± 1.24	29.20± 0.90	<0.001

Both groups C and D had a mean number of follicular cells of 18.94 and 29.2, respectively. A comparison of multiple groups revealed a significant difference between Groups A and D. Insignificant difference was found between groups A and C (Table-II).

Table-II: Multiple Comparison of Mean Number of Cells in the follicles among Control and Experimental Groups of Albino Rats (n=40)

Number of Cells in the Follicles		
Groups	Mean difference	p-value
A vs B	28.26	<0.001
A vs C	1.58	0.291
A vs D	11.84	<0.001
B vs C	24.86	<0.001
B vs D	16.42	<0.001
C vs D	10.26	<0.001

DISCUSSION

The thyroid gland is one of the vital endocrine organs in the body as it regulates important physiological functions such as energy metabolism in adults.^{11,12} Thyroid hormones play important roles in various human physiological processes, controlling carbohydrate, lipid, and protein metabolism. DEHP causes oxidative stress in the thyroid gland and disrupts the thyroid hormone homeostasis in the body.^{13,14}

The present study showed that DEHP causes severe vacuolization in the thyroid glands. Lysosomes secrete acid phosphatase, which causes colloids to dissolve, resulting in the formation of vacuoles.¹⁵ These results align with the work done by Cheon *et al.* documented that DEHP is an endocrine disruptor that alters uterine histology by causing vacuolization.¹⁶ Selenium more significantly improved it in Group-C as compared to Zinc in Group- D. These results were in accordance with a study done by Pinar Erkekoglu *et al.* who reported that selenium supplementation at the dose of a 1 mg/kg diet for five weeks reduces

inflammation in the liver caused by DEHP.¹⁷ Our results are supported by Ruggeri *et al.* who observed that Selenium reduces oxidative stress in thyrocytes against H₂O₂.¹⁸ Results obtained in this study were largely consistent with those reported by Alimohamady *et al.* who reported that Zinc plays an important role in protecting the thyroid gland by detoxifying oxygen-free radicals.¹⁹

In the present, DEHP caused an increased number of follicular cells in Group-B. Several studies reported that DEHP causes hyperplasia in the thyroid gland by causing oxidative stress.²⁰ Nurhayat Barlas observed similar findings, who reported follicular and colloidal degeneration by phthalates in the thyroid gland.²¹ In accordance with our results, Kim *et al.* reported disorganization of follicular cells with hypertrophy and hyperplasia of thyrocytes by DEHP at the dose of 150 mg/kg for 90 days in rats.²² Ye *et al.* and Liu *et al.* observed similar results after administration of DEHP at a dose of 500mg/kg/day for 30 days in rats.^{23,24} They reported that the number of thyroid follicular epithelial cells was increased in treated animals which is an indicator of hyperactivity of the gland. In the current study, the number of follicular cells was more significantly improved by Selenium as compared to Zinc, as an insignificant difference was found between Groups A and C. In accordance with our findings, a study conducted by Vasiliu *et al.* documented that Selenium supplementation helps restore the normal thyroid morphology in iodineinduced autoimmune thyroiditis in rats.²⁵

CONCLUSION

Selenium and Zinc have a protective effect on thyroid histomorphological changes caused by DEHP, but Selenium shows better conservation than zinc on thyroid histology.

Conflict of Interest: None.

Author's Contribution

NH: Direct contribution, SA: Reviewer of manuscript/supervisor, MB: Data analysis, TK: Formatting of manuscripts, HB: Review of Manuscript, SSB: Manuscript writing.

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Protective Effect of Selenium and Zinc Against

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