ORIGINAL ARTICLE

ALLEVIATION OF ARSENIC INDUCED THYROID DYSFUNCTION AND BODY WEIGHT ALTERATIONS BY CURCUMIN

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Background: Arsenic is notorious for being used in many homicidal cases. This study was designed to observe the toxic effects of low dose of arsenic on thyroid gland and body weight and its amelioration by curcumin. **Method:** Thirty healthy female Sprague Dawley rats (body weight ~230 g) were procured from The National Institute of Health Islamabad. After one week of acclimatization, animals were randomly divided into three groups (n=10 each), e.g., Control group (C), Experimental group-1 (E1) and Experimental group-2 (E2). The duration of the experiment was one month. Weight of the animals was checked before and at the end of experiment. All the animals were continued on standard diet and distilled water during experimental period. E1 was given 10 µg/10 mL of arsenic by oral gavage daily. E2 was given 10 µg/10 mL of arsenic along with Curcumin (50 mg/Kg body weight/day). At the end of experiment, the rats were euthanized to draw the blood and removal of thyroid gland. Serum Triiodothyronine, Tetraiodothyronine and Thyroid Stimulating Hormone were measured by ELISA. Histological changes were observed after haematoxylin and eosin staining. Statistical analysis was done on SPSS-22 and $p \leq 0.05$ was considered significant. **Results:** The histological findings of thyroid gland, body weight and serum T3, T4 and TSH in E1 exhibited abnormal results. E2 group showed the protecting role of curcumin suggesting protective role of curcumin. **Conclusion:** Curcumin has protective effects against arsenic induced thyroid disruption and body weight alterations.

Keywords: Arsenic, Curcumin, Triiodothyronine, Tetraiodothyronine

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INTRODUCTION

Arsenic is a metalloid that exists in nature in various oxidation states, e.g., +3 (arsenite), +5 (arsenate), -3 (arsenide). The +3 (arsenite) state of arsenic is most toxic and highly reactive than other states. It is notorious for being used in many homicidal cases and is called 'king of poisons'.[1] Some natural sources (weathering of minerals) and anthropogenic sources (use of arsenic in agriculture to kill un-wanted weeds and in many industries, e.g., ceramic industries and in manufacturing weapons) are increasing arsenic concentration in the drinking water.[2] Arsenic can enter the human body through various routes, including drinking water, skin contact, air inhalation, and dietary intake.[3]

Arsenic has been proven to be an endocrine disruptor.[4] One of the major component of endocrine system is Thyroid gland and its secretions (T3, T4 and Calcitonin). This gland is under control of TSH and TRH released from Pituitary gland and hypothalamus respectively.[5] Higher concentrations of arsenic in drinking water cause thyroid dysfunction along with imbalance in its hormones.[6]

Curcumin, the principal polyphenol is derived from the rhizomes of *Curcuma longa* (commonly known as turmeric).[7] Its ameliorative effects against arsenic induced hepatotoxicity, nephrotoxicity, genotoxicity, neurotoxicity and reproductive toxicity have already been affirmed.[8,9]

This study was designed to observe the toxic effects of low dose of arsenic on thyroid gland and body weight and its amelioration by curcumin given for a brief period of time.

MATERIAL AND METHODS

This laboratory based experimental study was conducted in the animal house of College of Physicians and Surgeon Pakistan (CPSP), Islamabad, after ethical approval from the Research Ethical Committee of CPSP Islamabad. Healthy female Sprague Dawley rats (n=30, body weight ~230 g) were procured from National Institute of Health Islamabad and kept under a standard cycle of 12-h light/darkness with food and water *ad libitum*. After one week of acclimation, the animals were divided into three groups (n=10 each) named control (C), experimental group 1 (E1) and experimental group 2 (E2). Arsenic for this study was bought from Hamza Enterprises Sadder Rawalpindi (LAB CHEM Lot No. 1406). Duration of the experiment was one month. The weight of the animals was checked before and at the end of experiment.

Control rats were on 10 mL of pure distilled water daily by oral gavage. After doing the pilot procedure, arsenic at a dose of 10 µg of sodium arsenite

dissolved in 10 mL of distilled water was given to group E1 by oral gavage daily. E2 was on 10 µg of sodium arsenite dissolved in 10 mL of distilled water along with Curcumin (50 mg/Kg body weight/day).

For hormonal assay, the blood from animals was drawn by single intra cardiac puncture and stored in gel activator vials and left for ~30 minutes in a thermocol with ice-packs. Serum was separated by centrifugation at 2,000 g for 10 minutes. Serum was collected in pre-labelled aseptic eppendorff tubes and stored at -20 °C until the hormonal assay by ELISA was performed.[10]

For estimation of serum Triiodothyronine (T3), rat ELISA kit by ELK biotechnology Cat # ELK8952 was used. The kit had sensitivity of 51.7 pg/mL and detection range=156.25–10,000 pg/mL. For serum Tetraiodothyronine (T4) estimation, rat ELISA kit by ELK biotechnology Cat # ELK056ES was used that had sensitivity 7 ng/mL and detection range 9.38–600 ng/mL. For Thyroid Stimulating (TSH), rat ELISA kit by ELK biotechnology Cat # ELK2283, was used that had sensitivity 0.071 ng/mL and detection range 0.16–10 ng/mL.

At the end of experiment, the rats were euthanized and dissected. Histological analysis of thyroid gland was done after Hematoxylin and Eosin (H&E) staining.[11] Four microscopic fields were evaluated from ten non-consecutive sections stained with H&E for each group under 10× magnification.

Data was expressed as Mean±SD on SPSS-23. The data was analysed by one-way ANOVA (for comparison between different groups) and Post hoc Tukey HSD test (for inter group comparison) and $p \leq 0.05$ was considered significant.

RESULTS

Control group showed normal histological structure, e.g., the gland consisted of follicles of different sizes, each surrounded by a single layer of regular cuboidal epithelial cells with intact basal lamina. Within these follicles, there was homogenous colloid present and the colour of cytoplasm was pink. The follicles were separated by capillary beds.

Experimental group E1 showed that the typical architecture of the thyroid gland was completely lost. Some follicles showed disruptions in their basal laminae, leading to their fusion with adjacent structures. Sizes of many follicles were reduced with atrophied epithelial lining and less colloid, while others were entirely collapsed. The apical membranes of some thyrocytes were also disrupted resulting in the presence of desquamated cytoplasm and nuclei within the follicles. The cytoplasm appeared less pink in colour.

The Experimental group E2 shoed improvement in the previously observed histological changes. The thyroid gland showed signs of returning to its normal architecture. A mixed pattern with few regular and majority having irregular follicular epithelium was observed. The size, shape and basal lamina of most of the thyroid follicles had returned to normal. Colloid staining densities varied, suggesting ongoing recovery. However, some follicles still exhibited disruptions. The colour of the cytoplasm appeared to be pinker.

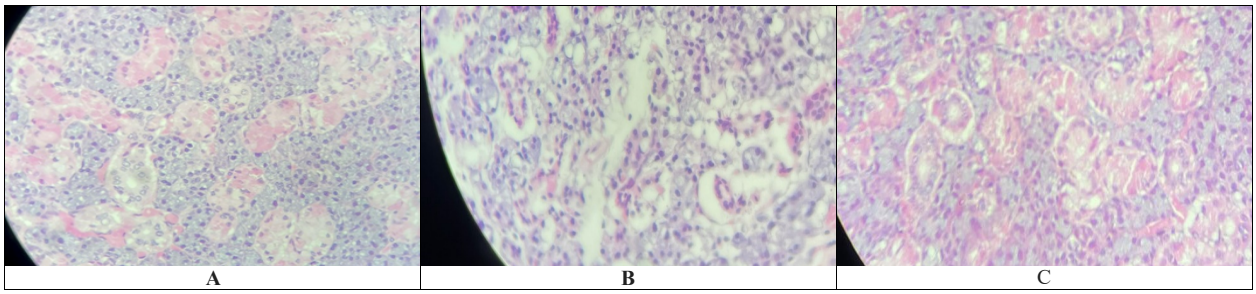

Figure-1: H&E stained section of Thyroid

A: The control group rats shows follicles lined with regular cubidal epithelium with intact basal laminae with colloid in centre and pink cytoplasm (10×).
B: E1 group rats shows the atrophied thyroid follicles with disrupted basal laminae. Less quantity of colloid with change in colour of cytoplasm (10×).
C: E2 group rats shows improvement as compared to E1. A mixed pattern of regular and irregular follicular epithelium and basal laminae. Colloid is clearly visible in most of the follicles with pinker cytoplasm (10×)

Results of one way ANOVA are expressed in Table-1. The weight of the control animals remained the same. The weight of group E1 was reduced significantly, and E2 group showed partial growth impairment ($p<0.05$) suggesting a potential protective role of curcumin against arsenic-induced toxicity.

Biochemical analysis of thyroid hormones showed that arsenic exposure in E1 reduced serum T3 and T4 and elevated serum TSH levels ($p<0.05$) indicating arsenic induced thyroid impairment Whereas, E2 had reversed these deranged values towards normal indicating the protective effects of curcumin against arsenic induced thyroid damage.

Table-2 is showing the results of Post Hoc Tukey HSD test indicating inter-group comparison. There were statistical significant differences among all the groups ($p<0.05$).

Table-1: Serum T3, T4, and TSH among control and experimental groups (Mean±SD)

Variables	Control	Experimental		
	Group (n=10)	Group-1 (n=10)	Group-2 (n=10)	*p*
T3 (pg/mL)	98.10±13.12	51.50±7.36	77.90±8.02	0.00
T4 (ng/mL)	50±8.11	9.5±3.1	22±3.1	0.00
TSH (ng/mL)	0.31±0.15	2.74±0.51	1.83±0.38	0.00
Body weight (g)	240.6±7.29	191.2±7.6	214.6±5.29	0.00

Table-2: Inter-group comparison by Post Hoc Tukey HSD test

Variables	Group *vs* Group		*p*
T3 (pg/ml)	Control	E 1	0.000
		E 2	0.000
	E 1	E 2	0.000
T4 (ng/ml)	Control	E 1	0.000
		E 2	0.000
	E 1	E 2	0.000
TSH (ng/ml)	Control	E 1	0.000
		E 2	0.000
	E 1	E 2	0.00
Body weight (g)	Control	E 1	0.000
		E 2	0.000
	E 1	E 2	0.000

DISCUSSION

The reason for the histological and biochemical effects of arsenic on the thyroid gland of E1 group animals lies in arsenic's ability to disrupt cellular processes and induce oxidative stress, inflammation, DNA damage, and alterations in cell signalling pathways. Arsenic interferes with normal cellular functions within the thyroid gland, leading to structural changes at the microscopic level. These changes can impair thyroid function and contribute to the development of thyroid-related diseases.[3]

The current investigations assessed that curcumin has protective role against harmful effects of arsenic on the thyroid gland due to its antioxidant, anti-inflammatory, and cytoprotective properties.[12] It reduces oxidative stress by inhibiting lipid peroxidation and augmentation of anti-oxidant enzymes, e.g., catalase, super-oxide dismutase, and glutathione peroxidase.[13] Therefore, supplementation with curcumin may help preserve the structural integrity and function of the thyroid gland in the face of arsenic exposure.[14] These results are similar to the study of Kamlesh *et al*.[15]

Decrease in body weight observed in E1 after exposure to 10 µg/10 mL solution of arsenic is likely attributable to the toxic effects of arsenic, which disrupt various metabolic processes associated with growth and development.[16] According to body surface area conversion formula, the human equivalent dose (HED) of 10 µg per 0.25 Kg of arsenic in rats is approximately 5.76 µg/Kg. Arsenic gets accumulated in muscle cells and adipocytes and stimulates lipolysis by G-protein coupled receptors and inhibits lipogenesis. It augments the release of adipokines from muscle cells, alters the metabolic capacity and bioenergetics of mitochondria that further causes weight loss.[17] Nevertheless, E2 group animals exposed to both arsenic and curcumin supplementation experienced a partial rescue from weight loss compared to those solely exposed to arsenic, indicating an effective mitigating effect of curcumin against arsenic-induced toxicity. The mechanism by which curcumin prevented arsenic-induced weight loss remains unclear; however, the high antioxidant content of curcumin likely contributed to scavenging free radicals generated by arsenic.[7] This perspective is supported by previous studies demonstrating the prevention of arsenic-induced weight loss by turmeric and *Phyllanthus emblica* leaf extract, both of which are known to possess ROS scavenging activity.[18,19]

Comparison between control group and experimental group 1 by Post hoc Tukey's test indicated that there is significant difference in the body weight and thyroid hormonal profile of E1 animals as compared to control group showing arsenic induced toxicity. The comparison between control group and E2 also showed a statistical significant difference which means that curcumin, in a dose of 50 mg/Kg body weight/day given for one month has ameliorated the toxic effects of arsenic but couldn't reduce arsenic induced thyroid dysfunction and body weight alterations towards control group values.

CONCLUSION

Low dose of arsenic given for a brief period of time is toxic for thyroid gland and its hazardous effects can be ameliorated by administration of curcumin.

IMPACT OF STUDY

The results of this animal-based experimental study can be extrapolated on human beings who are already exposed to arsenic contaminated drinking water.

FUTURE RECOMMENDATIONS

These results of inter-group comparison lead towards certain future recommendations regarding replication of this study design with possible alterations, e.g., dose, duration and route of administration of curcumin.

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Contribution of Authors:

AM: Animal Dissection and tissue processing
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WH: Blood sampling and ELISA
HAB: Dose adjustment of arsenic and curcumin, Statistical analysis
SM: Histological analysis of tissue
MY: Dose adjustment of arsenic and curcumin, Statistical analysis

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