Original Research Article

Comparative Effects on Wistar Rat's Thyroid Gland, Serum Selenium and Iodine Concentration of Bisphenol A, Carbimazole and Thyroxine

Abstract

The comparative effects of Bisphenol A (BPA), carbimazole and thyroxine on thyroid gland were investigated in 40 Wistar rats assigned as untreated controls, BPA (20 µg/kg/day), carbimazole (5 mg/day), and thyroxine (25 µg/day) in drinking water. BPA and carbimazole caused hypothyroidism within 30 days, and changes in thyroid follicles were correlated with alteration in serum thyroid hormones, selenium and iodine concentrations. Thyroxine was found to induce hyperthyroidism as evidenced by elevation of T_4 and T_3 with a significant decrease of TSH. No animal death occurred during the experimental period.

Keywords: BisphenolA, Thyroid hormones, Thyroxin, Carbimazole, Selenium, Iodine.

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1. Introduction

Bisphenol A (BPA), an estrogenic endocrine-disrupting chemical, used to manufacture polycarbonate plastic and epoxy resins which are used as coatings in the food-packing industry. Polymerization of epoxy resin reactions were known not be fully complete, and that a considerable proportion of unreacted epoxy compounds can be recovered from food packed in containers lined with these plastics [1], [2]. BPA can be released into the environment during the manufacturing process and by leaching from the final products [3], [4], [5]. The migration of cured resin components into foods has also been reported. Unreacted epoxy compounds are thought to be toxic due to their alkylating properties [6]. Into the environment it can be detected in air, soil and aquatic ecosystem [7], and for all these reasons it is not surprising that it has been identified also in human tissues and fluids. BPA has well characterized estrogenic and other endocrine disrupting activities that are mediated via multiple molecular mechanisms, mainly nuclear receptor signaling pathways [8]. There is evidence for an anti-thyroid hormone effect of BPA

leading to the reduction of the thyroid hormone (TH) mediated gene expression by enhancing the TH receptor (TR) interaction with a transcriptional co-repressor [9]. THs regulate a variety of biological processes associated with metabolism, energy provision, development, somatic growth, and reproduction in vertebrates and, thus, effects of EDCs on the thyroid system may pose a hazard to human and wildlife health [10], [11]. The objective of this study was to evaluate the effects of low dose of BPA on thyroid structure and hormones, serum selenium and iodine concentrations as compared to exogenous hypothyroid (carbimazole) and hyperthyroid (thyroxine) induction.

1.1Objective

The objective of this study was to evaluate the effects of low dose of BPA on thyroid structure and hormones, serum selenium and iodine concentrations as compared to exogenous hypothyroid (carbimazole) and hyperthyroid (thyroxine).

2. Materials and methods

The present study was carried out in the Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, El Neelain, University, Sudan, after getting approval from Scientific Research Ethical Committee. Forty Wistar rats were obtained from the Faculty of Pharmacy University of Khartoum, reared within the premises of the animal house under 12 hours photoperiod with standard feed and drinking water provided *ad libitum* before the commencement of experimental feeding. Room temperature was maintained at 25±2 0C at adequate house ventilation. Then the animals were randomly allotted into four groups 1, 2, 3, and 4 each of ten rats (5 males and 5 females). Group 1 was designated as the control group. Extra Pure (97%) Bisphenol A powder (Sangon, China) was thoroughly dissolved in distilled water and rats of group 2 received this test chemical by oral gavage dose at 20µg /kg body weight/day. Group 3 (same as group 2) received carbimazole at (5 mg/day). Group 4 was given thyroxin at (25 µg/day) for four weeks' period.

2.1 Data collection

2.2 Serum analysis

After the end of the experimental period, rats of the control and treatment groups were anaesthetized with diethyl ether and humanely slaughtered. Blood was collected at slaughter in clean sterile vials and sera were separated thereafter to be analyzed for the thyroid hormones, Thyroxin (T_4), Triiodothyronine (T_3) and Thyroid Stimulating Hormone (TSH) according to Aviva Systems Biology [12]. Selenium and iodine concentration were analyzed using the Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)-mayo clinic laboratory [13].

2.3 Histopathological methods

Necropsy was conducted to identify gross lesions and specimens of thyroid gland were immediately being collected immediately after slaughter of rats, fixed in 10% neutral buffered formalin and embedded in paraffin wax, sectioned at 5m and stained with Hematoxylin and Eosin (H & E) [14].

2.4 Statistical analysis

Mean values of Thyroxin (T4), Triiodothyronine (T3), Thyroid Stimulating Hormone (TSH), serum selenium and iodine concentration, were compared using student's t-test [15].

3. Result

3.1 Clinical observations

The control Group 1 remained clinically normal throughout the experimental period. On the fourth day of the experiment, rats of group 2 showed nervous signs and in appetence. on the last day of experimental period one rat from groups 2 died.

3.2 Serum thyroid hormones concentration

Changes in the concentration of T3, T4 and TSH were presented in Table 1. By the end of week 4 (experimental period) the concentration of T3 and T4 were lower ($P<0.05$-0.01) in groups 2 and 3 received BPA at 20µg /kg body weight/day and carbimazole at 5 mg/day, respectively. Significant increase ($P<0.05$-0.01) of the two hormones was observed in group 4 given thyroxine at 25µg/day than the control rats of group 1. TSH concentration decreased ($P<0.01$) in groups 4 while a significant increase ($P<0.05$) was observed in group 2 received 20µg /kg body weight/day. TSH of group 3 was not significantly increased, but the value was a little bit higher the control.

Table 1: Changes in serum Thyroxine (T_4), Triiodothyronine (T_3) and Thyroid Stimulating Hormones (TSH)

Group No.	Dose	T3 ng/dL	T4 ng/dL	TSH µIU/mL
1	**Control**	107.60±0.89	109.40±0.67	0.038±0.17
2	**BPA (20µg/kg/day)**	87.00±0.21**	92.50±0.65**	0.045±0.32*
3	**Carbimazole (5mg/day)**	83.00±1.20**	85.50±0.32**	0.039±0.56NS
4	**Thyroxine (25µg/day)**	125.50±0.99**	112.50±0.47*	0.026±0.12**

NS = not significant, *Denotes mean values significant at (P<0.05), **Significant= (P<0.01).

3.3 Concentrations of selenium and iodine

Changes in the concentrations of serum selenium and iodine of the test groups and the control group are presented in Table 2. The concentration of selenium was lower (P<0.05) in group 4 than the controls. No significant differences were observed in the selenium of group 2 and 3 and the iodine concentration of all the test group.

Table (2): Concentration of serum selenium and iodine in BPA, carbimazole and Thyroxine dosed Wistar rats

Group	Administered dose	Selenium µg/ml	Iodine µg/ml
1 Control	Nil	0.42±0.03	0.16±0.04
2	Bisphenol A 20 µg/kg/day	0.38±0.05NS	0.14±0.02NS
3	Carbimazole 5 mg/day	0.39±0.02NS	0.10±0.014NS
4	Thyroxine 25 µg/day	0.29±0.014*	0.092±0.02NS

NS = not significant, *Denotes mean values significant at (P<0.05).

3.4 Thyroid histopathology

In the control group 1, the thyroid gland was normal. In the thyroid gland of a female rat of group 2, the majority of the thyroid follicles varied greatly in size and colloid contents with lymphocytic infiltration. Damaged follicles were also seen (Fig1). In some instances, the thyroid follicles showed hyperplasia (Fig 2) of the epithelial cells and aggregates of lymphocytes in the interstitial. In Male rats of group 2 the thyroid follicles also varied in size and colloid content, but the damaged follicles were less marked (Fig. 3). The thyroid gland of carbimazole- treated rats was affected. Some of the thyroid follicles become dilated with varying amount and densely stained colloid and many other appeared smaller in size. aggregates of lymphocytes were also observed (Fig.4). In a female rat of the same group 3, some of the thyroid follicles wee enlarged with slightly stained colloid with other follicle appeared smaller in size with lymphocytic infiltration in the interstitium (Fig.5). The administration of thyroxine also affected the structure of the thyroid gland of the treated rats of group 4. Follicular damage and diffuse hyperplasia were observed in the thyroid glands of both male and female rats of group 4 received thyroxine at 25 µg/day as evident in Fig 6. Follicular damage and accumulation of lymphocytes and fibroblasts were observed also in a male rat given the same above dose of thyroxine

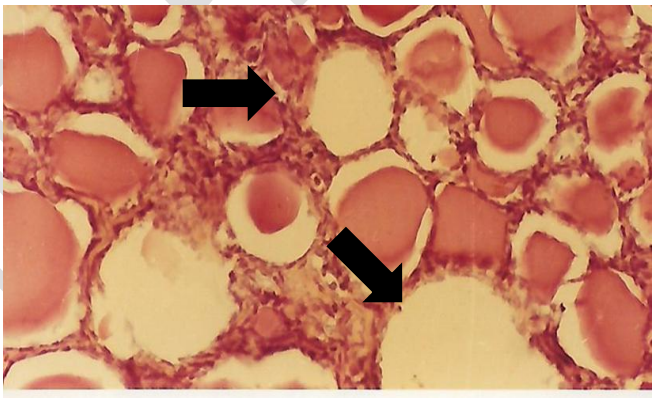

Fig 1: Thyroid gland of a female rat received BPA 20µg /kg body weight/day showing follicular damage with little lymphoid cell accumulation. H & E ×120

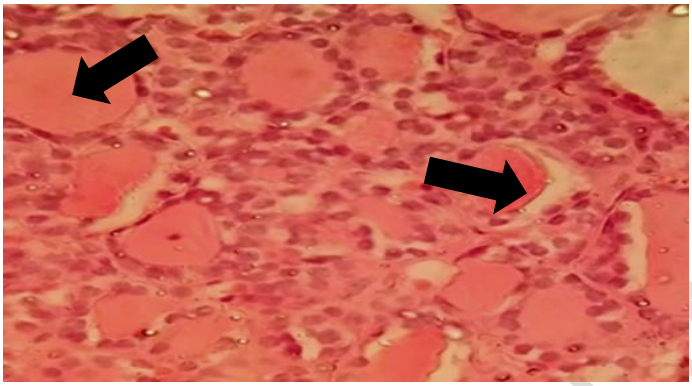

Fig 2: Hyperplasia of the thyroid follicular cell in female received BPA at 20µg /kg body weight/day. H & Ex120.

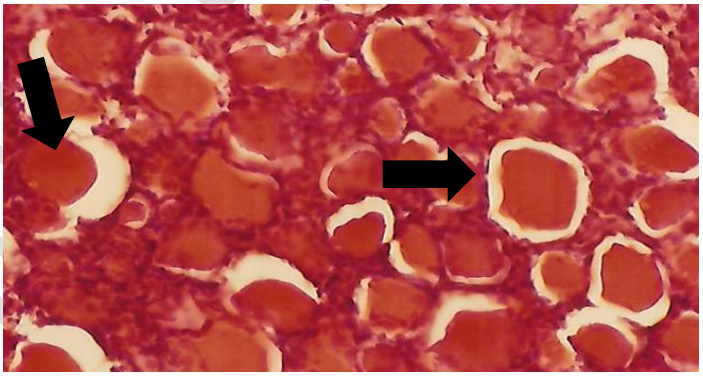

Fig 3: Thyroid gland of a male rat of group 2 given BPA at 20µg /kg body weight/day depicting damage to follicles and follicular epithelium with aggregates of lymphocytes. H & E X 120

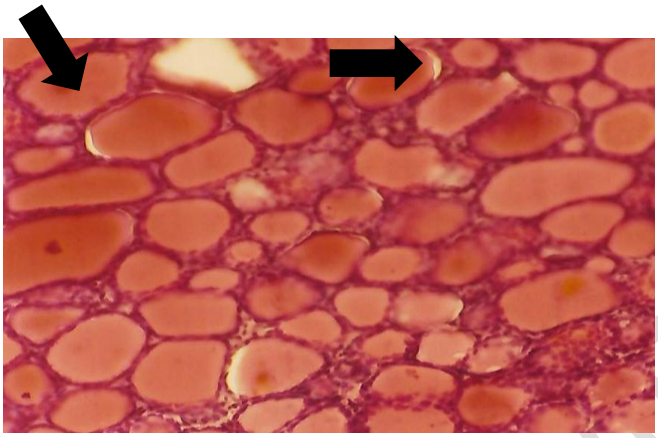

Fig. 4: Small and elongated follicles with densely stained colloid in a thyroid gland of a male rat of group 3 given carbimazole at 5 mg/day. H & Ex120.

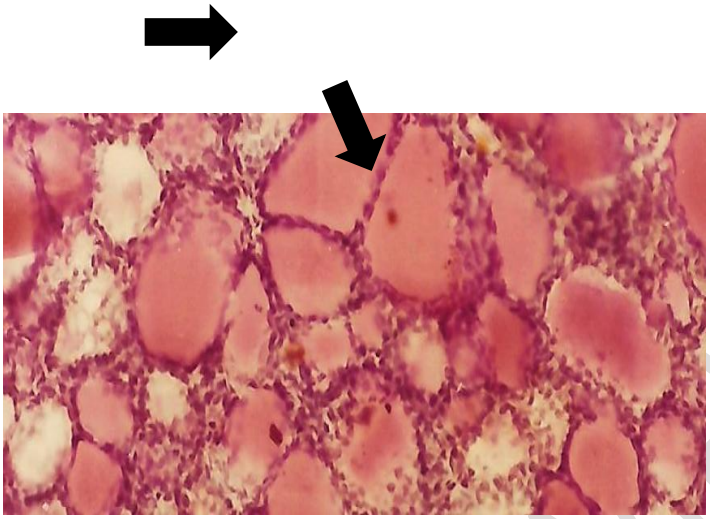

Fig. 5: Lightly stained large follicles and small sized ones with interstitial lymphocytic infiltration in a female rat of group 3 received carbimazole at 5 mg/day. H & Ex120.

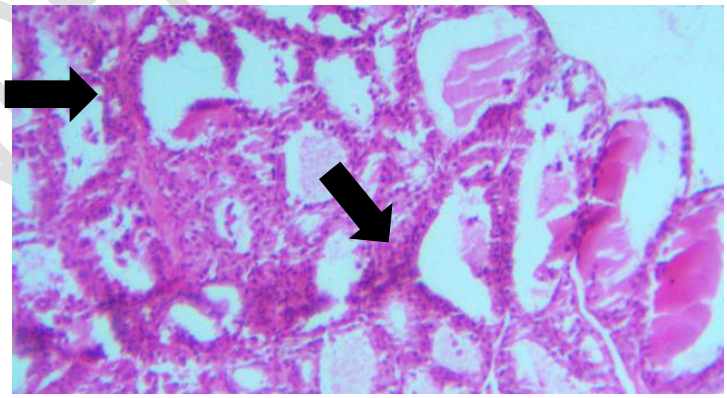

Fig. 6: Diffuse thyroid hyperplasia and damaged follicles in a female rat given thyroxine at 25 µg/day. H & Ex120

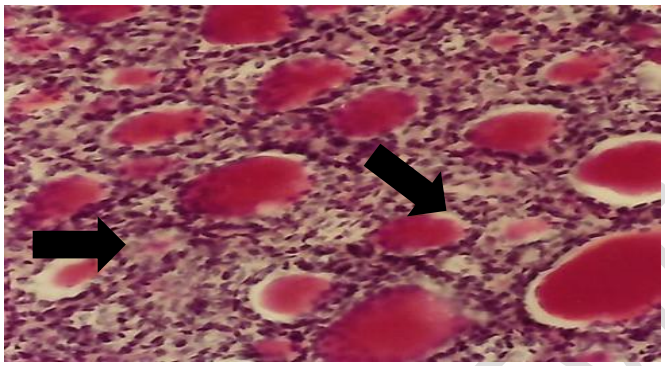

Fig. 7: Damaged thyroid follicles with lymphocytic accumulation and deposition of some fibroblast and degeneration of follicular epithelium in a male rat of group 2 given thyroxine at 25 µg/day. H & Ex120.

4. Discussion:

Endocrine Disrupter Chemicals (EDCs), which modify natural endocrine function, have emerged as a major public health issue due to their potentially disruptive effects on physiological processes, particularly through direct interaction with steroid hormone receptors [16]. Some of these EDCs were reported to be responsible for disruption of thyroid system function [17] [18]. Thyroid hormones (thyroxine T_4 and triiodothyronine T_3) are essential for normal behavioral, intellectual, and neurological development. Congenital hypothyroidism if left untreated causes irreversible mental retardation. Even mild maternal thyroid deficiency during pregnancy could cause retarded neurological development of the child [19]. The results of the present study indicated the anti-thyroid effect of low dose of BPA for Wistar rats of both sexes (20 µg/kg/day) where the thyroid gland microscopic and functional alteration were observed. That was evidenced by the decreased concentration of T_4 and T_3 with a significant increase of TSH in

BPA, degeneration of the thyroid follicles with lymphocytic infiltration. However, neoplasia was not detected in this group. *In vitro* and *in vivo* (zebrafish) models were used by Genticore [20] to examine the effects of BPA in regulating the expression of the genes involved in thyroid hormone synthesis and of their transcriptional regulators at BPA low doses. In both systems, altered expression in the genes involved in thyroid hormones synthesis was detected. Also, the direct effect on thyroid follicular cells, which are affected by very low amount of BPA, was observed as well, thus introducing a new vision into the action of BPA-induced deregulation of physiological processes and the molecular pathways showing its biological activity. BPA was found to inhibit TR-mediated transcription by acting as an antagonist and suppressed transcriptional activity that is stimulated by thyroid hormone (T3) in a dose-dependent manner.

Many human and animal BPA administration experiments have been conducted. It was administered directly to animals, and thyroid hormone values were detected. In adult rats, oral BPA exposure at 40 mg/kg for 15 days, increased T4 levels [21]. Whereas neonatal subcutaneous exposure to BPA at 2.5 to 6.2 mg/kg for 10 days, decreased T4 levels and increased TSH levels in adulthood [22]. The inconsistent results of these experiments might be attributed to different doses, routes of exposure to BPA in addition to environmental condition which can induce stress to experimental animals.

Experimentally, suppression of hormone production has been the base for studying thyroid dysfunctions and changes in the metabolism and body development [23] [24], or thyroxin administration in high doses inducing hyperthyroidism status [25] [26]. However, most studies suggested the use of traumatic protocols and methods for hormone dosage or the induction methodology using rats as animal model. For unknown reasons resistance to induction of hypothyroidism in mice was described [27].

The induction of hypothyroidism (carbimazole) and hyperthyroidism (Thyroxine) in treated rats was confirmed by the functional and histopathological alteration of the thyroid gland at the end of the experimental period. Carbimazole is an antithyroid drug similar to methimazole i.e. a prodrug converted to methimazole after administration. The mode of action of carbimazole is to act as an inhibitor for thyroid peroxidase (TPO) and decreases incorporation of iodide into tyrosine molecules. It also inhibits coupling of mono-iodinated and di-iodinated residues to form T4 and T3. Carbimazole has been the drug of choice in some patients because it may have few side effects, such as less frequent Gastrointestinal tract problems [28]. Thyroidal effects produced

by carbimazole, at the end of the experimental period, were similar to those produced by BPA i.e. hypothyroidism.

L-thyroxine, used for induction of hyperthyroidism in this study, is a synthetic form of the thyroid hormone thyroxine and is often used for the treatment of hypothyroidism and thyroid hormone deficiency. It has the ability to lower the thyroid-stimulating hormone (TSH), a hormone that is considered goiter-inducing [29] [30].

The relation between selenium and iodine has been well studied by [31] [32]. The authors found that selenium is needed for hepatic conversion of thyroxine (T_4) to 3,3,5-triiodothyronine(T_3) and that type 1 iodothyronine deiodinase, identified as a selenocysteine containing enzyme, catalyzes deiodination of (T_4) to biologically active thyroid hormone T_3 and thus play an important role in thyroid hormone metabolism in rats and cattle.

5. Conclusion

The results of the present study indicated the hypothyroid effect of low dose of BPA for Wistar rats of both sexes (20 µg/kg/day) where the thyroid gland microscopic and functional alteration were observed. That was evidenced by the decreased concentration of thyroid hormone and increase of thyroid stimulating hormone.

Ethical approval

Animal ethic Committee approval has been collected and preserved by the author.

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