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Effects of varying concentrations of iodine intake on thyroid gland in rats

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Abstract

Although iodine is an essential micronutrient needed for the creation of thyroid hormones, too much of it can cause systemic problems and thyroid malfunction. Elevated iodine levels can impact multiple organs and interfere with thyroid function. This study investigates the effects of low and heigh dose of iodine intake on thyroid function, histopathological changes and biochemical alterations in rats. six healthy control rats (150–200g) as group 1, iodine solution was ingested orally to 6 rats at dose 500 µg / kg/day as group 2 and iodine solution at dose (1500 µg /kg/day) was orally administered to 6 rats as group 3. This procedure was performed for 14 days. At the end of this period thyroid function parameters as T3, T4 and TSH levels were recorded in blood samples, and thyroid tissues were also examined histopathologically. Results demonstrated that excessive iodine intake led to thyroidal stress, alterations in thyroxine (T4) triiodothyronine, (T3) levels and TSH, hyperthyroidism in low dose and hypothyroidism in heigh dose group were observed, and deteriorative changes in thyroid tissue were detected. These findings emphasize the possible dangers of excessive iodine intake and highlight the importance of maintaining balanced iodine levels to avoid thyroid and systemic disorder.

Keywords: Excess iodine, thyroid hormones, T3, T4, TSH, hypothyroidism, hyperthyroidism.

Introduction: **Iodine is an fundamental trace element that plays a role in preserving thyroid health. The thyroid gland depends on iodine to manufacture the hormones T3 (triiodothyronine) and T4 (thyroxine), which adjust critical processes such as metabolism, growth, and tissue development (Zimmermann & Boelaert, 2015). Significant thyroid abnormalities can result from both iodine excess and shortage, underscoring the significance of preserving a healthy balance (Leung et al., 2012).**

One of the earliest trace components to be recognized as vital was iodine. It was founded in the 1920s to be a fundamental part of the thyroid hormone thyroxine (T4), which is essential for healthy growth and metabolism. It was rapidly recognized as a constituent of 3,5,3'–triiodothyronine (T3), a key regulator of critical cellular functions. Thyroid hormones play a greater part in the maturation of the organism as a whole and are essential for the suitable growth and development of tissues as the central nervous system (Freake, 2000). They are enhancing cells' ability to synthesize energy and use oxygen, which helps to maintain the body's metabolic rate balanced (Freake, 2000) .

The normal value of iodine in the human body mainly depends on the context in which it is determined, such as urinary iodine levels, nutritional intake, or thyroid iodine content. Total Iodine in the Human Body about 15–20 mg of iodine, with approximately 70–80% reserved in the thyroid gland (National Institutes of Health, NIH, 2024). Urinary Iodine Concentration (UIC) is the most used indicator of iodine status in normal Range is 100–199 µg/L (sufficient iodine intake). this procedure reflects recent iodine ingestion as more than 90% of dietary iodine is released in urine (World Health Organization, WHO ,2007).. nutritional Iodine Intake in Adults: 150 µg/day. These values are founded on recommendations to prohibit iodine deficiency disorders (U.S. Institute of Medicine, 2001). finally Thyroid Iodine material 10–15 mg of iodine, which it uses to develop thyroid hormones T3 and T4 (National institutes of health, NIH ,2024). These values represent typical iodine levels in a healthy human, essential for maintaining metabolic and thyroid health.

On the other hand, there are more and more reports about the negative consequences of consuming large amounts of iodine. Food, drinking water, treatment, and iodized salt or iodinated oil are all sources of excessive iodine exposure (Yang et al., 2006). In addition to goiter, consuming too much iodine can increase the risk of thyroid cancer, iodine–induced autoimmunity, hyperthyroidism, and hypothyroidism (Prakash R ,2005).

This research aims to investigate the complex interplay between iodine intake and thyroid health, focusing on the delicate balance required to prevent thyroid dysfunction. By analysing the consequences of both iodine concentrations low and heigh dose, the study attempts to emphasize the importance of iodine intake level consuming for optimal thyroid function and overall health.

Methods of Research and the tools used

Animals :Male adult rats weighing between 150 and 200 grams were obtained from the Theodor Bilharz Research Institute's Schistosoma Biological Supply Program (SBSP) in Cairo, Egypt. Randomly divided into three groups, containing 6 rats each. All rats stay for two weeks, at a temperature 25±2°C, humidity 50±5% and light and dark cycle. The rodents were given tap water and a typical rat diet.

Experimental procedure :**Random placing of six animals each in 3 groups A, B, and C was done. Group A served as control, Group B administered oral dose of low dose of iodine 500ug/kg/day for 14 days, and Group C was administered heigh dose of iodine 1500 µg /kg/day orally for 14 days.**

The rats were placed in an anaesthetic box filled with ether vapor on cotton wool at the box's base after being fasted for the entire night. Following the sacrifice of the animals (control and treated), blood samples were taken for T3, T4, and TSH biochemical analyses. The animals were dissected, and thyroid exposed. The thyroid from each rat was taken and processed for histological examination.

The biochemical analyses involved collecting blood samples, placing them in a clean, dry centrifuge tube for each rat, centrifuging the samples for 15 minutes at 5000 r.p.m., and then freezing the serum at –20°C for further analysis.

Our data were analyzed using SPSS 18.0. An analysis of variance, or ANOVA, was used in one way to determine group differences. The Post Hoc Tukey test was utilized to observe any variations in means between the groups. The Fisher exact test and Chi–square test were used to observe the relationship between groups' qualitative factors. A statistically significant p–value was defined as < 0.05.

Results of Research

Biochemical result

The results showed statistically significant differences in (T3, T4, TSH) hormones levels between the treatment groups and the control group in a rat model (table 1).

The Table show that: T3 (Triiodothyronine) Thyroid hormone in low–dose iodine group (500 µg/day) showed a significant comparing with control group ($p < 0.05$). But , high–dose iodine group (1500 µg/day) showed a significant decrease in T3 levels comparing with control ($p < 0.05$).

Also, T4 (Thyroxine) Thyroid hormone on low–dose iodine group (500 µg/day) showed a slight increase in T4 levels comparing with control group. But, high–dose iodine group (1500 µg/day) showed a significant decrease in T4 levels comparing with control ($p < 0.05$).

The pituitary gland secretes the hormone known as TSH (thyroid stimulating hormone): TSH levels were significantly lower in the low–dose iodine group (500 µg/day) than in the control group ($p < 0.05$). However, TSH levels were significantly higher in the high–dose iodine group (1500 µg/day) than in the control group ($p < 0.05$).

Graphs:

The graphs clearly showed the trends mentioned above, with colored bars representing the mean hormone levels in each group (yellow for the control group, blue for the low dose, and green for the high dose).

Histopathological results

The thyroid gland of control rats (group 1) is surrounded by a thin connective tissue capsule that divides the gland into lobules made up of follicles. The interlobular connective tissue contains lymphatic vessels, veins, and arterioles. Perifollicular capillary capillaries normally fill with blood. Thyroid follicles come in a variety of sizes and are primarily spherical or slightly oval in shape. Larger follicles are seen on the periphery of the core zone of particles, while medium and tiny follicles predominate. Large follicles have somewhat flattened intracellular thyrocytes, which are primarily cubic in shape and line the follicular lumen. One layer of follicular thyrocytes is present. The follicles' space is filled with a somewhat variable oxyphilic colloid, which typically fills the entire cavity. Cell nuclei are compact and spherical (fig. 1).

Under a microscope, the thyroid glands of rats given a low dose of iodine (group 2) showed areas of epithelial growth and a little buildup of colloid in the follicles. The colloid itself is reticulated and has a faint hue. Additionally, there were "empty" follicles. The majority of follicles range in size from medium to small. Moderate focal lymphoid infiltration was found at various particle locations (in the interfollicular stroma) (Fig. 2).

Histological indicators of autoimmune disease of glandular tissue were markedly more prominent in rats treated with a high dosage of iodine (group 3). Proliferation of interlobular connective tissue layers was observed. There is more noticeable focal lymphoid infiltration (Fig. 3). Increased manifestations of hypotrophy. There were indications of noticeable growth in the follicular epithelium. More frequently, papillary growths occupy the entire follicular cavity and are distributed more uniformly among the many follicles. Additionally, the thyroid tissue in the neck is encircled by many lymphocytic nodules (fig. 3, 4, 5).

Effect on external appearance

It was observed that the Group 3 of rat that were treated with high dose of iodine experienced noticeable hair loss. This is a symptom of hyperthyroidism caused by excess iodine (fig. 6).

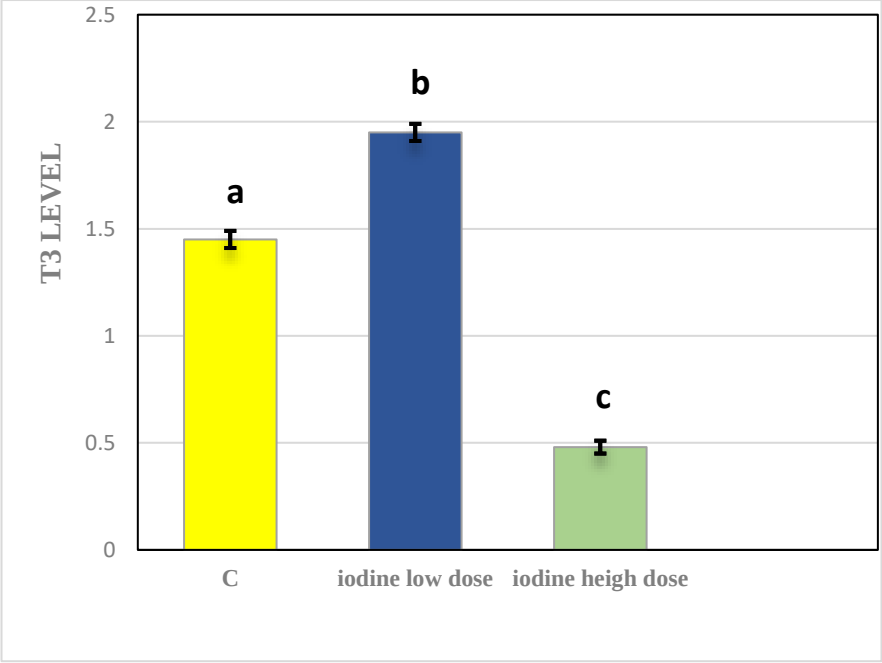
Table (1): Effect of daily oral administration of oral iodine (500 µg/kg /day) and (1500 µg/kg /day) for 14 days on T3 (ng/ml), T4 (µg /dl), TSH (ng/ml) level in rat model.

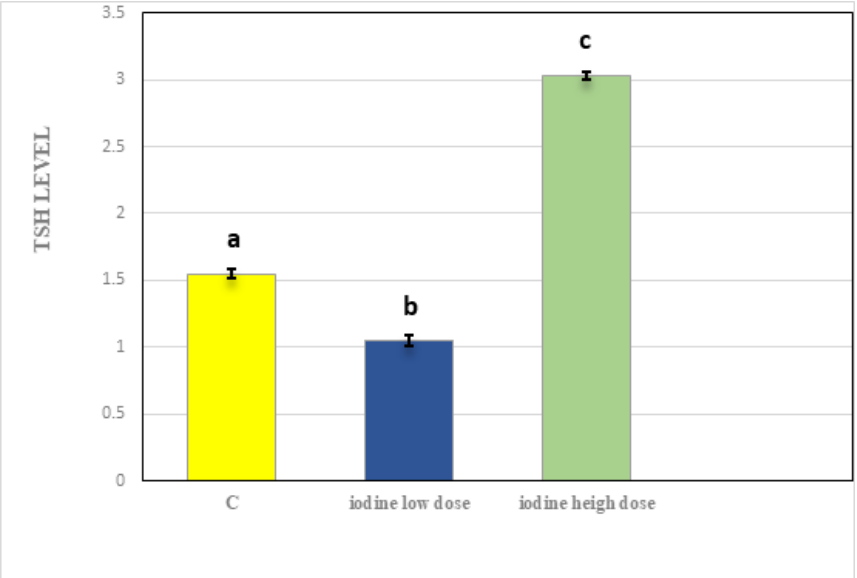
parameter	Control	Iodine low dose	% D	Iodine heigh dose	% D	p-value
T3	1.45^{a} ±0.04	1.95^{b}±0.04	34.48%	0.48^{c}±0.03	-66.89%	0.000
T4	4.12^{a} ±0.03	4.23^{b}±0.03	2.67%	3.65^{c}±0.04	-11.41%	0.000
TSH	1.55^{a} ±0.04	1.05^{b}±0.4	-32.25%	3.03^{c} ±0.5	95.48%	0.000

Significance between groups at p value < 0.0

Statistically significant means (P value < 0.05) are given different letters and statistically non-significant means are given the same letter.

D: Percentage difference [(Treated value – Control Value) / Control Value] x 100 %




TSH LEVEL
3.5
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iodine low dose
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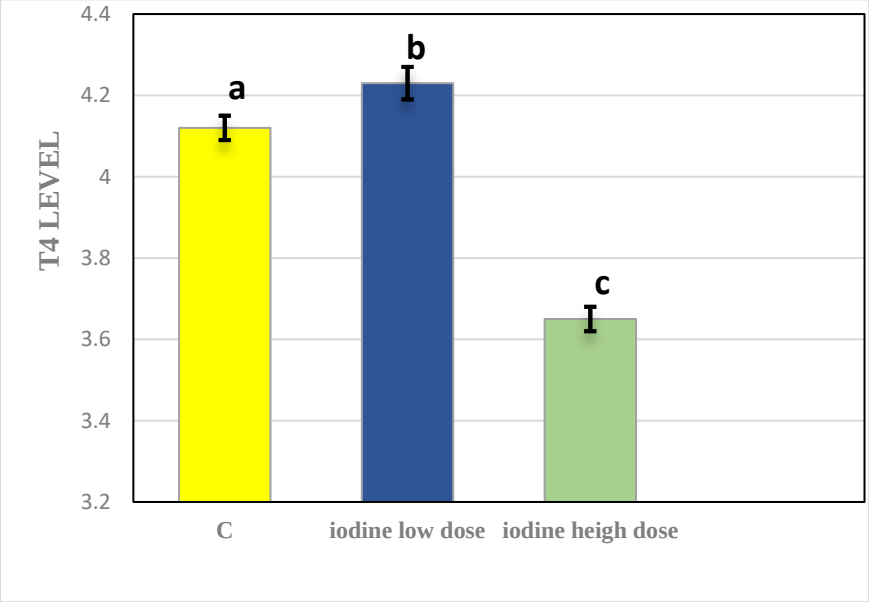
T4 LEVEL
4.4
4.2
4
3.8
3.6
3.4
3.2
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b
c
C
iodine low dose
iodine heigh dose

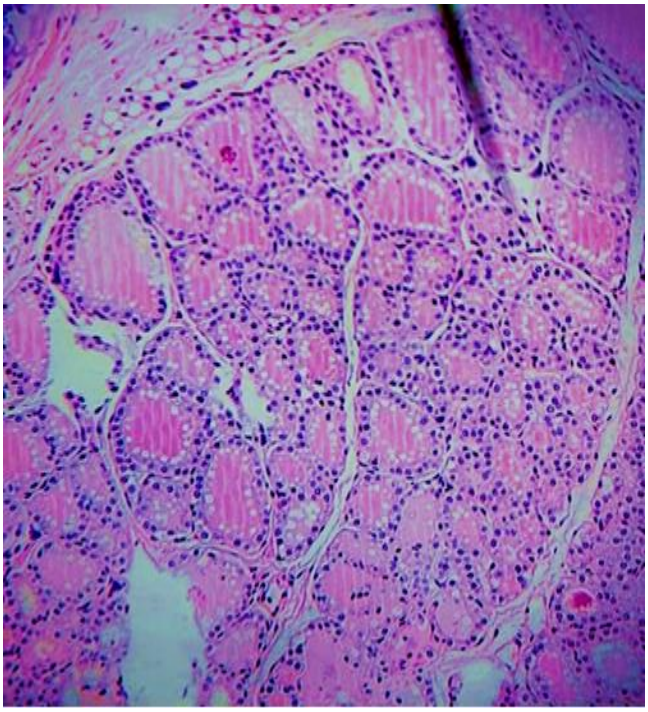


Fig. (1) Thyroid gland of a control rats showed normal follicular structure of the tissue with oval to rounded thyroid follicles lined with flat to cubical follicular cells. Homogenous acidophilic colloid fills the follicles. A cluster of parafollicular cells is seen between thyroid follicles. Blood capillaries surround the follicles and are found between parafollicular cells. Hematoxylin–eosin, ×200

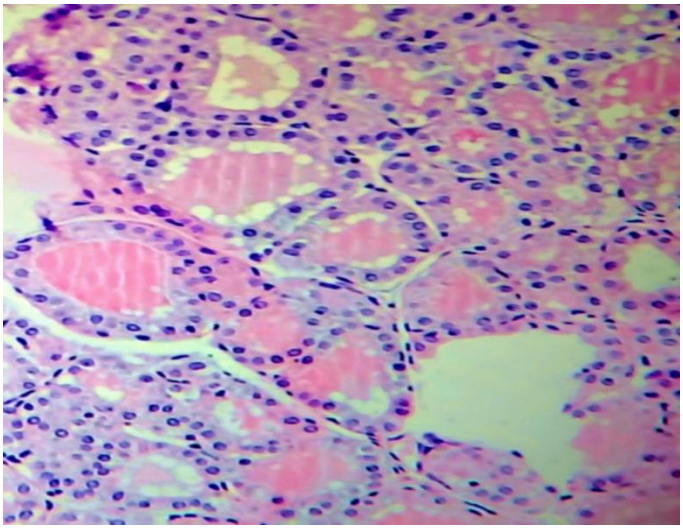
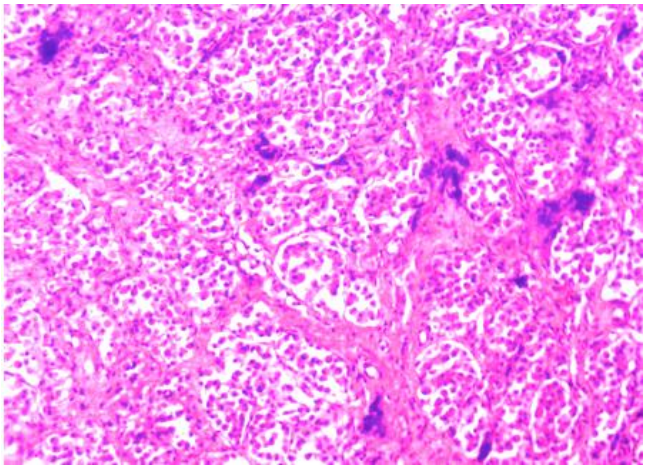
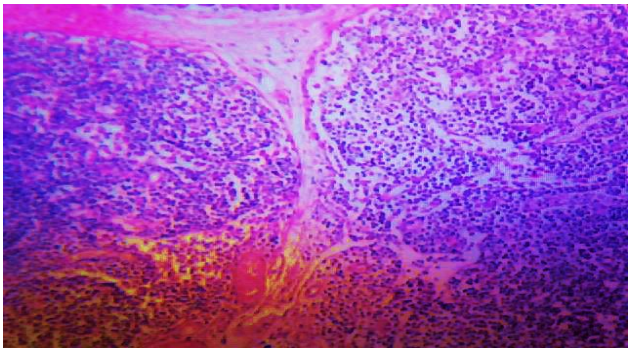


Fig. (2) Rats given a low dose of iodine displayed considerable localized lymphocytic infiltration of the stroma and uneven epithelial growth in several follicles. Hematoxylin–eosin, ×250

. Fig. (3) Rats given a high dose of iodine displayed thyroid glands with substantial epithelial proliferation, cell desquamation, lack of colloid, connective tissue layer fibrosis, and increased lymphoid infiltration foci.Rats given a high dose of iodine displayed thyroid glands with substantial epithelial proliferation, cell desquamation, lack of colloid, connective tissue layer fibrosis, and increased lymphoid infiltration foci. Hematoxylin–eosin, ×200





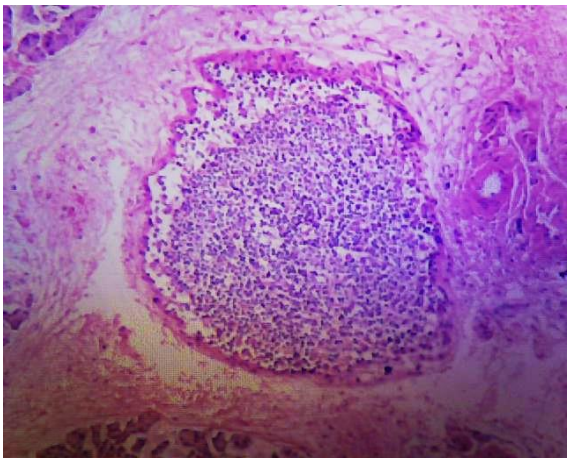
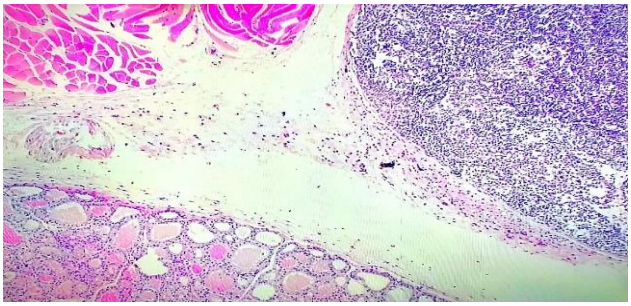
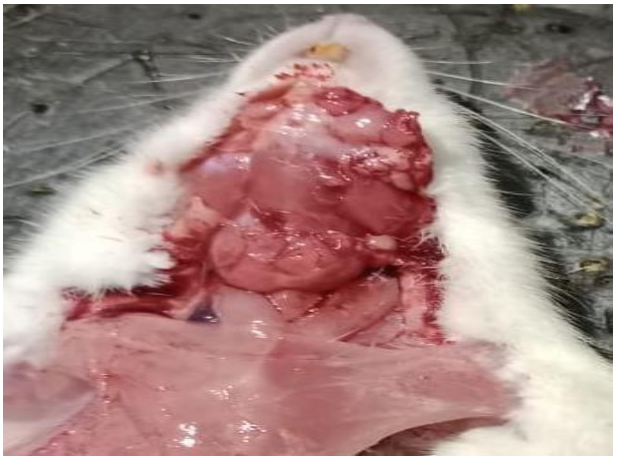


Fig. (4) many adenomatous nodules are seen in the hyperplastic background on rats treated with high dose of iodine.





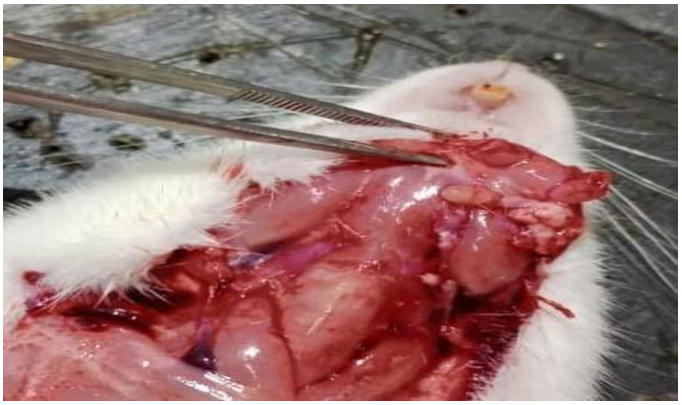


Fig. (5) many adenomatous nodules are seen on rat treated with high dose of iodine

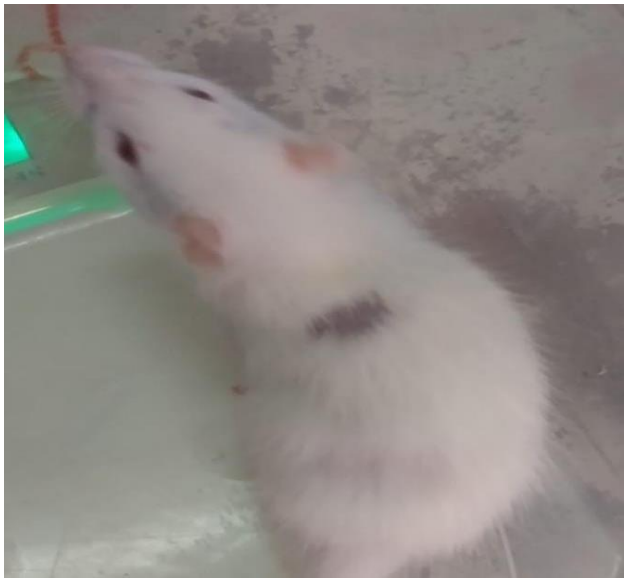


Fig. (6) noticeable hair loss of rats treated with high dose of iodine

Discussion

Discussion: thyroid is located in the inferior, frontal neck and is in charge of iodine homeostasis and thyroid hormone production and secretion. About 90% of the thyroid generates thyroxine (T4), an inactive thyroid hormone, and 10% produces triiodothyronine (T3), an active thyroid hormone. Peripherally, inactive thyroid hormone is converted to either activated thyroid hormone or an inactive thyroid hormone substitute (Maggie Armstrong, 2023). Serious disruptions in the chemical and morphological structure of several organs can result from thyroid abnormalities (Sarkar et al., 2018).

Thyroid function ultimately depends on the proper iodine supply to the gland; physiologically, thyroid function is controlled by two pathways, i.e., the hypothalamic–pituitary–thyroid axis regulation and thyroid autoregulation (Braverman LE, & Roti E., 1996). Iodine, the primary component of thyroid hormone, is an important trace element of the human body and plays a significant role in the growth, progress, and metabolism of mammals. The effect of iodine on the body is U–shaped, and both shortage and excess can harm the body (Zimmermann, 2009). Thyroid autoregulation will initially be activated as a result of changes in iodine intake. One efficient strategy to maintain healthy thyroid function is to regulate the manufacture of thyroid hormone precursors and the conversion of T4 to T3. Wistar rats have an adaptation mechanism that allows them to tolerate high levels of iodine (Chen et al., 2005). Although the effects of excess iodine on thyroid function vary depending on the type, thyroid dysfunction was generally reported to develop when iodine was provided at doses ten times the physiological need (Chen et al. 2005).

In the present study, rats were exposed to 500 and 1500 µg/kg iodine for 2 weeks, The current study demonstrated that excess treatment by iodine caused significant rise in concentration of T3andT4 on the low dose of iodine but show decreasing of them in heigh dose group, and an increase in TSH plasma levels. The current study examines structural and functional changes in the thyroid and investigates the effect of these changes on serum thyroid hormone concentrations. These changes included a decrease in the whole thyroid size, formation of markedly dilated follicles with a flat epithelium, unevenly shaped follicles, there was insignificant changes in all measured parameters in comparison to the control group.

According to our findings and those of other studies, amiodarone's high iodine burden may hasten the onset of hypothyroidism in euthyroid patients who already have chronic autoimmune thyroiditis. Hypothyroidism in these patients typically lasts a long time (Bogazzi et al., 2012). Iodine shortage is a well–documented cause of thyroid–linked

disorders, including goiter and hypothyroidism, both of which can lead to a cascade of health problems if left untreated (Hetzel & Delange, 2004). On the other hand, extreme iodine consumption, though less common, can also disrupt thyroid function (Markou et al., 2001). Conditions such as iodine toxicity, hyperthyroidism, or paradoxical hypothyroidism may arise from high iodine intake, emphasizing the need for careful observing of iodine levels (Dunn & Delange, 2001).

Furthermore, conditions like Hashimoto's disease, an autoimmune disorder in which the immune system attacks thyroid tissue, complicate the situation and frequently result in chronic hypothyroidism (Weetman, 2003). Excessive iodine can also cause oxidative stress, which exacerbates thyroid damage, especially in people who already have thyroid dysfunctions like hypothyroidism (Venturi, 2001). Oxidative stress can cause cellular damage, inflammation, and weakened hormone production (Con et al., 1993).

Additionally, according to some research, the thyroid produces MIT and DIT by concentrating iodide from the serum, oxidizing it at the apical membrane, and binding it to tyrosyl residues in thyroglobulin. Then, while remaining inside the thyroglobulin molecule, two DIT residues marry to form T4, or one DIT and one MIT combine to form T3 (Dunn JT, & Dunn AD, 2000). MIT and DIT provide around two–thirds of the iodine in thyroglobulin, despite being dormant precursors of thyroid hormones (Dunn JT & Dunn AD, 2001). Consequently, changes in the proportions of these iodotyrosines will reflect the effects of varying iodine intake levels on the thyroid. Therefore, in order to clarify and examine the effects of iodine consumption on thyroid function and its mode of action, a practical and trustworthy method for quantifying them in the thyroid is needed.

The hypothalamus is the starting point for thyroid hormone neural control. Thyrotropin–releasing hormone (TRH) is secreted from the hypothalamus into the anterior pituitary gland through the hypothalamic–hypophyseal portal system. Thyroid–stimulating hormone (TSH) is released by the anterior pituitary's thyrotropin cells in response to stimulation by TRH (Braun, Schweizer, 2018).

TSH and a free thyroxine (free T4) test are the first–line methods to check for any thyroid abnormalities. These establish whether the thyroid gland, pituitary, or hypothalamus are the core or peripheral sources of the problem. The thyroid gland is not producing enough thyroid hormones when primary hypothyroidism is suspected. As a result, free T4 levels will be lower and TSH levels will be suitably higher. According to our findings, free T4 levels are abnormally elevated in primary hyperthyroidism, while TSH levels are appropriately decreased. The TSH test can

be used to diagnose Hashimoto thyroiditis or Graves' disease, respectively (Karapanou et al., 2017).

A clinically significant disorder linked to iodine excess is the Jod–Basedow phenomenon, were individuals with underlying thyroid pathologies, such as nodular goiter, experience hyperthyroidism after a sudden increase in iodine intake (Roti & Vagenakis, 2000). This phenomenon is especially common in regions where iodine deficiency is common, and dietary supplementation efforts are implemented (Stanbury et al., 1998).

Yang et al. (2006) found that when the iodine dose reached 3000 µg/l, the mice's serum T4 level rose and their serum T3 level considerably reduced when compared to the control group. However, at a dose of 1500 µg/l, iodine had no discernible effect. The primary cause of this alteration might be the suppression of D1–deiodinase activity, which lowers the production of T3 from T4 (Wang et al., 2006). This is evident from the fact that the expression of the 5'–deiodinase gene was significantly reduced. This could be interpreted as a successful strategy to shield the organism from harm brought on by excessive T3. According to recent research, iodopeptides are produced that temporarily block the creation of thyroid peroxidase (TPO) proteins and mRNA, which in turn prevents thyroglobulin iodination. The main mechanism for removing excessive amounts of iodide and preventing the thyroid from producing large amounts of thyroid hormones is the Wolff–Chaikoff effect (Wang et al., 2006).

Conclusion: To date, published reference intervals for histological and clinical biochemistry data in Wistar rats in this point are few. In this study, we clearly demonstrate significant changes of thyroid function and tissue by compare the results of the experimental group which take excess doses of iodine with the control group in pre–clinical toxicology studies using Wistar rats as an animal model.

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