Section A-Research paper

Biogenic Selenium Nanoparticles Synergizes Levothyroxine in the Treatment of Hypothyroidism at the Level of Genes Expression

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Abstract

Hypothyroidism is the sufficient thyroid hormone production by thyroid gland. Selenium is the specialist elements required for thyroid antioxidant activity. The present study attempted to investigate role of SeNPs in synergizing Levothyroxine in treatment of hypothyroidism. Biogenic selenium nanoparticle synthesized using sarcomona (SC-SeNPs) Fifty female Wister rats of 3-4 months aged were equally and randomly divided into 5 groups, 1st control group, the 2nd , 3rd, 4th, 5th groups were hypothyroidim by orally intubation of 0.02% Methimazole daily for 30 days. After the verification of hypothyroidism, experimental rats were treated with Sc-SeNPs (0.1mg selenium /kg/ day) as T1, or Levothyroxine (0.9 µg/100g BW/day) as T2, or SC-SeNPs (0.1mg selenium /kg/ day)+ Levothyroxine (0.9 µg/100g BW/day) as T3, in addition to hypothyroidism untreated group for 28 days. Results show that there was significant decrease ($p \leq 0.05$) in thyroid hormone T3, T4 and TSH of the hypothyrodism groups comparing with control. Changes of TSH,T3 and T4 in blood were the best in T2 and T3. Moreover, the histomorphological measurement for thyroid follicles diameter significantly decreased in hypothyrodism in addition to Enlargement of thyroid gland due to hypertrophy of most of thyrocytes, narrowing the follicle lemen and absence of thyroglobulin colloid, all these changes were ameolarated in treated groups. On the level of gene expression Sc-SeNPs alon or combined with Levothyroxine significantly decrease ($p \leq 0.001$) upregulation of thyroid peroxidase (TPO) and Glutathione peroxidase (GPX1) genes expression in hypothyroidism thyroid gland.

Keywords: Primary Hypothyroidism; SC-SeNPs; Levothyroxine; Thyroid peroxidase; Glutathione peroxidase.

Introduction

Thyroid hormones; thyroxine (T4) and triiodothyronine (T3) synthesized and secreted by thyroid gland regulate their synthesis by a negative feedback on the major thyroid stimulus hormone, the thyroid stimulating hormone (TSH) at level of hypothalamus and pituitary (Chiamolera and Wondisford, 2009, Ibraheem et al., 2018, Rustam and Hassan, 2021). Hypothyroidism is defined as failure of the thyroid gland to produce sufficient thyroid hormone to meet the metabolic demands of the body. Untreated hypothyroidism can contribute to many disorders; hypertension, dyslipidemia, infertility, cognitive impairment, and neuromuscular dysfunction (Gaitonde et al., 2012). The prevalence increases with age, and is higher in females than in males (Boucai et al.,

2011).The most common symptoms of hypothyroidism in adults are fatigue, lethargy, cold intolerance, weight gain, constipation, change in voice, and dry skin, but the clinical presentation can include a wide variety of symptoms that differ with age, sex, and time between onset and diagnosis (Carlé et al., 2015, Carlé et al., 2016, Hassan and Ahmed, 2021). Furthermore, patients with hypothyroidism have a higher prevalence of cardiovascular risk factors increased vascular resistance, decreased cardiac output (Gao et al., 2016). And often have features of metabolic syndrome, including hypertension, increased waist circumference, and dyslipidemia (Tiller et al., 2016).

In normal thyroid follicles were thyroid hormones are synthesis, both reactive oxygen species and antioxidant activity in restricted balance(Sinha et al., 2015). The synthesis of thyroid hormones by thyroid follicles cells required constant amount of reactive oxygen species (ROS) for iodination of thyroxin in production of thyroid hormones. The iodide ion (I-) should be oxidized by H2O2 dependent thyroxine peroxidase enzyme (TPO) to form highly reactive iodine (I+) that covalently binds to carbon 3 or carbon 5 of tyrosine residues on thyroglobulin to create monoiodinated tyroside (MIT) and diiodinated tyrosine (DIT) residues. TPO is also responsible for conjugation or coupling of MIT and DIT to yield triiodothyronine (T3), and thyroxine (T4), which are the two forms of active thyroid hormone (Ruf and Carayon, 2006),. Because of this ROS role in thyroid follicular cells function in production of thyroid hormones, the thyroid is highly exposed to oxidative damage. In front of this thyroid must effectively defense by antioxidant system regulate ROS production and scavenging. Among, the antioxidant enzymes the glutathione peroxidase. In hypothyroidism whereas thyroid hormones are reduced depress the antioxidant enzymes activity (Kochman et al., 2021, Villanueva, 2013). Glutathione peroxidase (GPx) and thioredoxin reductase, are selenoproteins, including play a role in protecting the thyroid gland with cellular antioxidative defense systems and redox control. The first identified selenoproteins were the GPx, which protect the cells against oxygen free radicals damage (Lubos et al., 2011).

Selenium is the second important micronutrient after iodin required for the antioxidant function and metabolism of thyroid hormones (Ventura et al., 2017). Maintaining a physiological concentration of selenium is a prerequisite to prevent thyroid disease and preserve overall health. Supplementation with selenium is determined by the form of this element, as organic or inorganic forms. As inorganic Se absorbed by plants is transformed into organic forms, which present a higher bioavailability (D'Amato et al., 2020), the inorganic mainly as sodium selenite and the organic form mainly as selenomethionin (Surai et al., 2018). Unfortunately, Se deficiency is a very common condition worldwide. Supplementation is possible, but as Se has a narrow safety level, toxic levels are close to those normally required for a correct need. Thus, whether the obtaining of optimal selenium concentration is desirable, the risk of dangerous concentrations must be equally excluded (Gorini et al., 2021). The performance response to organic selenium supplementation is influenced by health status and environmental impact (Verma et al., 2012). The available evidence from trials does not support routine selenium supplementation in the standard treatment of patients with autoimmune thyroiditis or Graves' disease (Winther et al., 2017). Despite recommendations only extending to patients with Graves ophthalmopathy, selenium supplementation is widely used by clinicians for other thyroid phenotypes. Ongoing and future trials might help identify individuals who can benefit from selenium supplementation, based, for instance, on individual selenium status or genetic profile (Winther et al., 2020a).

The biosynthesis of Selenium nanoparticles provide organic selenium source with biocompatibility, bioavalibility and low toxicity. For the synthesis of SeNPs, researchers have mostly reported the use of biogenic/biotic reduction using various reducing and stabile agents (Ahmed et al., 2016, Wali, 2019, Al-Kurdy,

2020). Although, literature referred to a slight bigger size than that synthesized by chemical methods, but still the biogenic method proved to be more efficient (Bano et al., 2022).

Experimentally hypothyroidism induced by methimazole as an medicamentous modeling of the thyroid hypofunction via inhibition of TPO in producing T4, T3. Relationship between thyroid hormones and serum selenium level is unresolved subject, due to the mechanism of hypothyrodism. There are little information about the quality and nature of the relation between selenium and TPO and GPx1 genes expression in thyroid gland tissue in experimental hypothyroidism whether it is inverse or direct. So this study was carried out to verify the Role of Biogenic SC-SeNPs in regulating the gene expression of TPO and GPx1 enzymes in thyroid tissue of hypothyroidism rats

Material and Method

Preparation of Se-nanoparticles:

The biogenic synthesis of Se-NPS was achieved using saccharomyces cerevisiae as reducing agent following procedure of Hariharan etal.,(2012) with some modifecations. Briefly: 100 gm of dried yeast dissolved in 1 L of deionized distilled water at 45C° in conical flask, and stirring with 50 gm sugar by 500 rpm stirring power for one hour, then, the yeast solution was mixed with 0.1 M of sodium hydrogen selenite (NaHSeO3) solution in 2:1 ratio v/v. Finally, the flask was stirred again on the magnetic stirrer (200rpm) at room temperature (22-24 C°) a for 24 hours nd monitoring the formation of red brick colour. which is the initial sign of the generation of SeNPs. The prepeared selenium nanoparticles (Sc-SeNPs) solution was Sonicated for 10 mins and washed twice with DDW after that dried the Sc-SeNPs solution in rotary evaporator. The dose of Sc-SeNPs was fixed according to the concentration of Se elements in the preapared nanoparticles by atomic absorption and 0.1 mg of Se /kg body weight (Wali and Alqayim 2019).

Experimental Animals

Fifty female Wister rats of age ranged between three to four months were randomly divided into two groups. The first group (10 rats) had daily been fed basal diet and given normal water for one month (control group), the second, third, fourth and fifth groups (40 rats) had daily been fed basal diet and given 0.02% Methemazole in drinking water to induce hypothyroidism. Hypothyroidism was verified by measuring thyroid tests hormones at 2nd and 4th weeks of methimazole treatment. After the verification of hypothyroidism, experimental rats were treated with Sc-SeNPs (0.1mg selenium /kg/ day)as T1, or Levothyroxine (0.9 µg/100g BW/day) as T2, or SC-SeNPs (0.1mg selenium /kg/ day)+ Levothyroxine (0.9 µg/100g BW/day) as T3, in addition to hypothyroidism untreated group.

Assessment of hypothyroidism

Blood sample collected for serum isolation and measuring thyroid hormones concentration, T3 and T4 measure in (CobasE411-Hitachi-1292-Japan), TSH concentration was measure follow the manufacture Sunlong Biotech Co., Ltd. Catalogue, Number: SL0684Ra ELISA kit**.**

Histomorphology and Histopathology change

At the end of the experiment thyroid samples were collected from euthanized rats and fixed in 10% formalin for tissue section stained with Hematoxylin and Eosin as routine stain and were examined by light microscopy and

microphotography has been done by using Future Win Joe microscopic camera, the images have been analyzed and scored by using Fiji image analyzer system.

Thyroid peroxidase and Glutathion peroxidase gene expression by thyroid cells were determined by quantitative Real Time PCR

Total RNA was extracted from thyroid samples (50mg) using Easy-spin™ (DNA free) total RNA extraction Kit, iNtRON biotechnology, South Korea. RNA isolated according to the manufacturer's protocol. The extracted RNA samples were quaintified (ng/µL), and qualified by reading the absorbance in at 260 nm and 280 nm in nanodrop spectrophotometer (Thermo.USA). We used Protocol of GoTaq® 1-Step RT-qPCR System for Real-Time qPCR (Gene expression assay). The relative expression of target genes in rat thyroid tissue was calculated (2^-ΔΔCT). That dependent on normalization of RT-qPCR (CT values) of target genes relatively to housekeeping gene (GAPDH) as reference gene in control and different treatment groups. The primers for Thyroid peroxidase gene (TPO) and glutathione peroxidase (Gpx1) and the hous keeping gene (GAPDH) in table 1.

Table 1. Primers of genes expression experiment

Gene	Primer name	5'-3'	Product (bp)	Accession number	Reference
TPO Thyroid peroxidase	TPO-RF	GCATGTATCATTGGGAAGCA	139	XM_032908631.1	Nazar et al., 2012
	TPO-RR	CGGTGTTGTCACAGATGACC			
Gpx1 Glutathione peroxidase	GPX1-RF	CAGTTCGGACATCAGGAGAAT	139	XM_032910620.1	Venardos et al., 2005
	GPX1-RR	AGAGCGGGTGAGCCTTCT			
GAPDH	GAPDH-F	ATGACTCTACCCACGGCAAG	89	NM_017008	Kunst et al., 2012
	GAPDH-R	CTGGAAGATGGTGATGGGTT			

Results

Assessment of hypothyroidism

As depicted in Table 1, the presence of hypothyroidism produced by methimazole, was substantiated by the occurrence of much higher TSH levels and lower T3,T4 concentrations in methimazole-treated rats starting from 2nd week and continued to 4th week. Hypothyroidism , as confirmed by serum T3, T4 values which were significantly lower than those of control rat for T3 and T4 and higher for TSH

Table 1. Assessment of hypothyroidism induced by methimazole for 4 weeks by thyroid function tests.

Group	T3(nm/l)		T4(nm/l)		TSH(ng/ml)	
	2 Weeks	4 Weeks	2 Weeks	4 Weeks	2 Weeks	4 Weeks
Control	0.93±0.06Aa	0.83±0.04Aa	36.62±6.12Aa	38.1±1.97Aa	2.36±0.29Ba	2.23±0.18Ba

Hypothyrodism groups	0.4±0.07Ba	0.4±0.07Ba	8.41±0.5Ba	8.1±0.6Ba	5.26±0.26Aa	5.22±0.25Aa
LSD	0.183		9.378		0.715	

Mean ± SEM. Capital letters indicates significant differences between groups ($p \leq 0.05$)

The present results illustrated in Table 2. revealed that the mixture treatment of levothyroxin and SC-SeNPs to hypothyrodism rats result the best thyroid hormones levels in compare with hypothyroidism treated with SC-SeNPs alone and untreated. Integrated Biomarkers response (figure 1) analysis revealed the more biomarker affected was T4

Table 2. Effects of Sc-SeNPS alone or in combination with levothyroxine in thyroid tests of hypothyroidism female rats for 4 weeks.

GROUP	T3(nm/l)	T4(nm/l)	TSH (ng/ml)
CONTROL	1.2±0.08A	35.49±2.89A	2.38±0.29C
HYPO	0.46±0.05B	7.06±0.77C	5.58±0.2A
T1	0.46±0.05B	14.37±2.76B	4.81±0.17B
T2	1.22±0.16A	36.39±2.23A	2.52±0.29C
T3	1.02±0.09A	30.26±2.57A	2.75±0.41C
LSD	0.27	6.99	0.84

Mean ± SEM. Capital letters indicates significant differences between groups ($p \leq 0.05$)

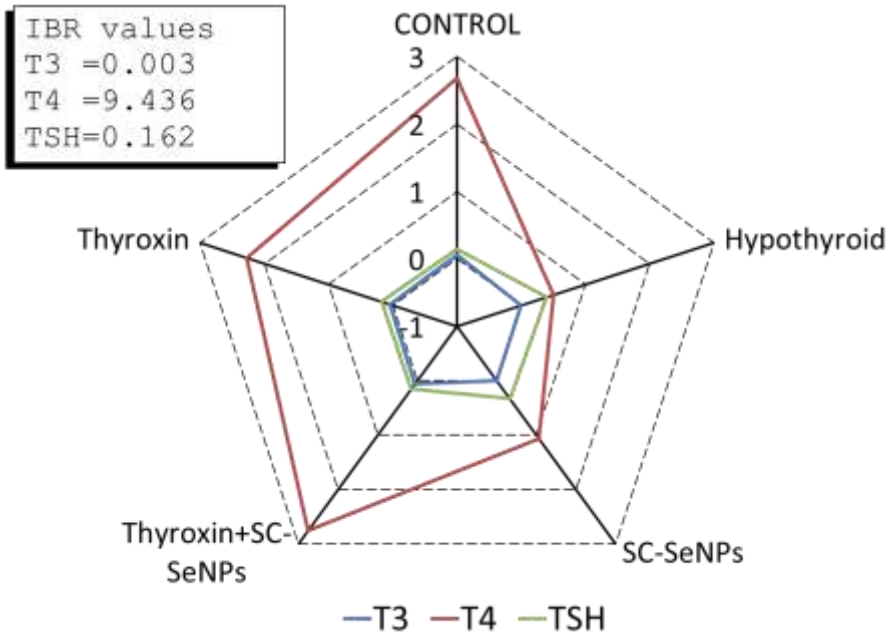

Figure 1. IBR (Integrated Biomarker Response) star plots for T3, T4 and TSH in control, hypothyroid , SC-SeNPs (0.1mg\kg.BW\Day) thyroxin (0.9 microgram/100g BW/day), thyroxin+SC-SeNPs (0.1mg\kg.BW\Day), and thyroxin (0.9 microgram/100g BW/day)

Table (3) Histomorphological measurement for thyroid follicles diameter in control and hypothyroidism rats for 4 weeks.

Follicle Diameter (micrometer)	
Group	Mean±SEM
Control	66.657±7.01A
HYPO	30.006±1.4D
T1	44.789±1.95 C
T2	55.189±2 B
T3	45.784±2.11C
LSD	10.49751

Mean ± SEM. Capital letters indicates significant differences between groups ($p \leq 0.05$)

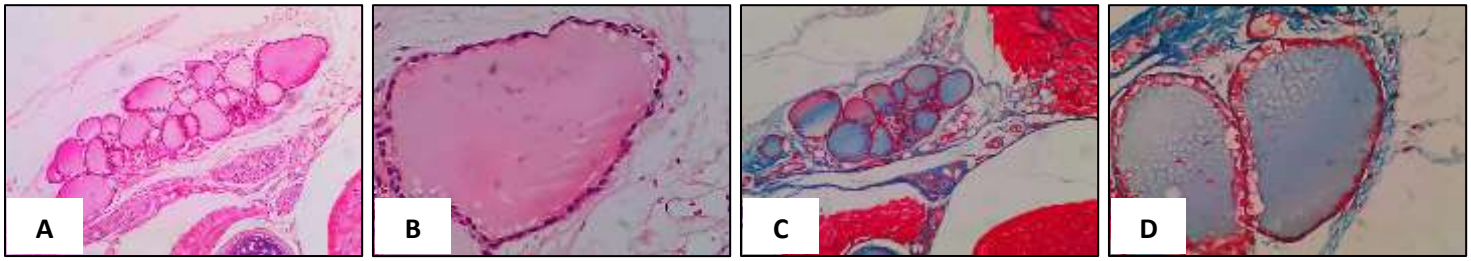

Figure 2. Photomicrograph of thyroid for control group rat. Normal histological architecture of thyroid, where the thyroid follicle was filled with colloid of thyroglobulin. A and C: 100x, B and D: 400x.

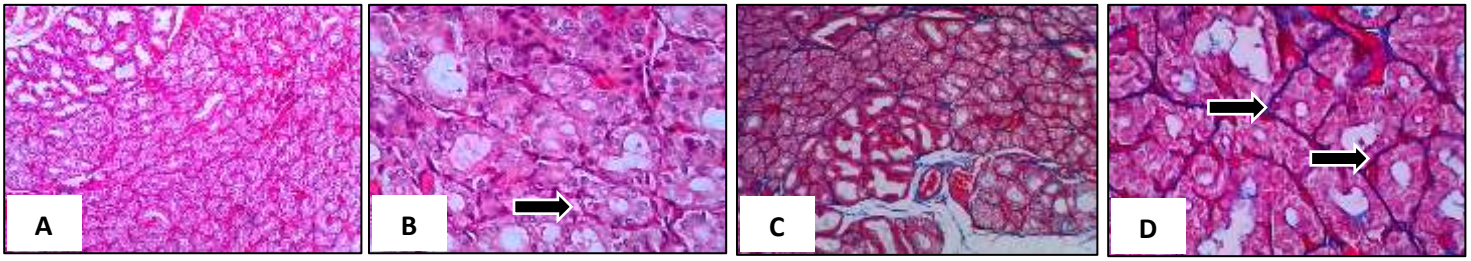

Figure 3. Photomicrograph of thyroid for hypothyroidism group rat. Enlargement of thyroid gland due to hypertrophy of most of thyrocytes (arrow) that led to narrowing the follicle lumen. Absence of thyroglobulin colloid, where the follicle lumen was empty. A and C: 100x, B and D: 400x.

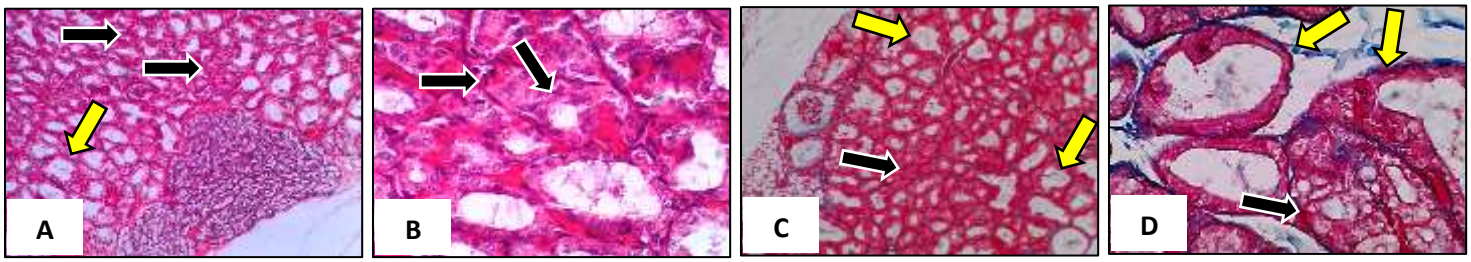

Figure 4. Photomicrograph of thyroid of SC-SeNPs treated group rat. Enlargement of thyroid gland due to hypertrophy of thyrocytes (black arrow) that led to narrowing the follicle lumen. However, some of thyrocytes showed normal thyrocytes appearance (yellow arrow). Absence of thyroglobulin colloid, where the follicle lumen was empty. A and C: 100x, B and D: 400x.

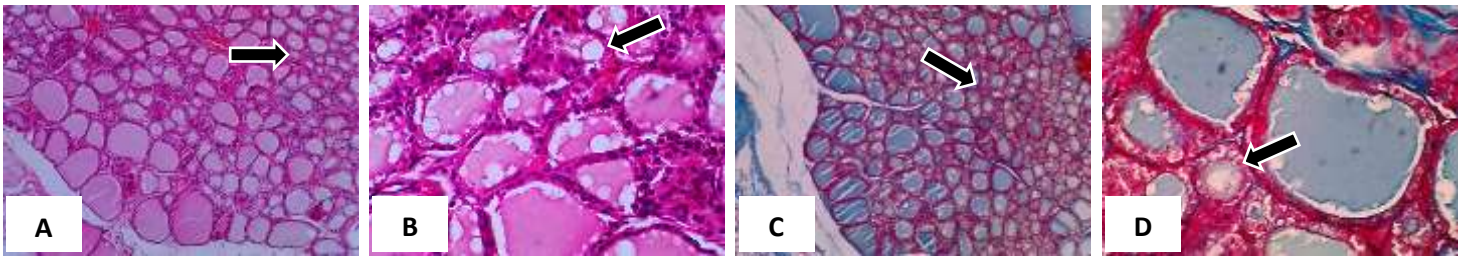

Figure 5. Photomicrograph of thyroid of levothyroxine treated group rat. Thyroid follicle was filled with thyroglobulin colloid, also thyrocytes showed normal appearance as simple cuboidal epithelial cells. However, narrowing of some of follicles (arrow) was observed. A and C: 100x, B and D: 400x.

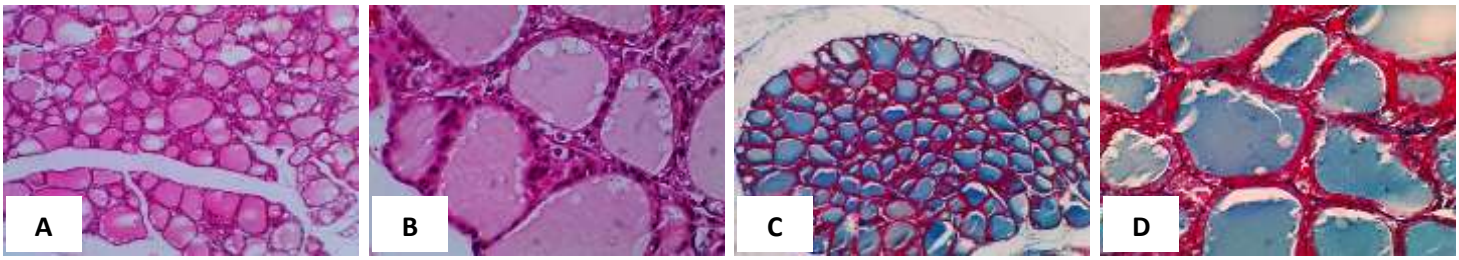

Figure 6. Photomicrograph of thyroid of levothyroxine and SC-SeNPs treated group rat .Thyroid follicle was filled with thyroglobulin colloid, also thyrocytes showed normal appearance as simple cuboidal epithelial cells. A and C: 100x, B and D: 400x.

Thyroid peroxidase and Glutathion peroxidase gene expression by thyroid cells were determined by quantitative Real Time PCR

The hypothyroidism clearly induced up regulation for TPO and Gpx1 genes expression in thyroid cells as illustrated in figure 2. On the other hand treatment of hypothyroidism with Sc-SeNPs alone or combined with levothyroxine were efficient in improvement these genes expression to semi normal levels

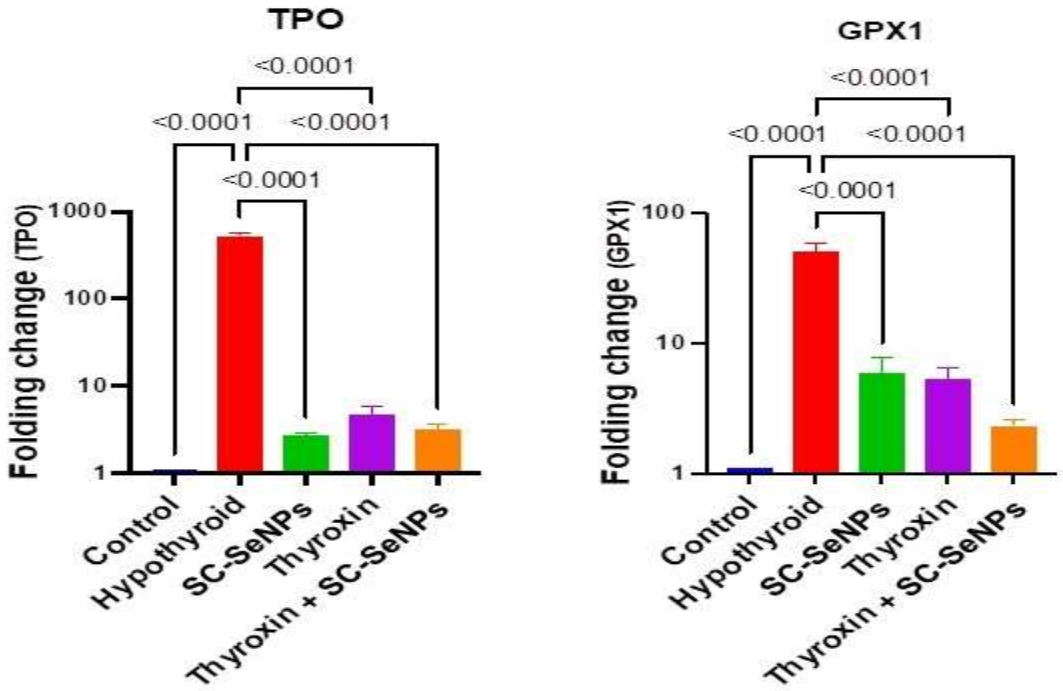

Figure 7. Effects of Sc-SeNPS alone or in combination with levothyroxine in Thyroid peroxidase and Glutathion peroxidase gene expression by thyroid cells of hypothyroidism female rats for 4weeks. P value 0.0001 for thyroid peroxidae (TPO) and glutathione reductase (GPX1).

Discussion

Primary hypothyroidism results from a low level of thyroid hormone due to destruction of the thyroid gland. This condition results in increased secretion and elevation of serum thyroid-stimulating hormone (TSH) levels. the presence of hypothyroidism produced by methimazole, was substantiated by the occurrence of much higher TSH levels and lower T3,T4 concentrations in hypothyroid than euthyroid. As mention by the results, methimazole induce clinical primary hypothyroidism is defined as thyroid-stimulating hormone (TSH) concentrations above the reference range and free thyroxine hormones concentrations below the reference range. (Chaker et al., 2017), within 2-4 weeks of adiminstration of metnmizole. This hypothyrodism induced by methimazole mediated by different mechanisms. Methimazole Inhibition of the enzyme thyroid peroxidase, that mediated oxidation and organification of iodine thus preventing the synthesis of thyroxine is the main described mechanism for the methimazole (Abraham and Acharya, 2010). Furthermore, methimazole suppress the mRNA expression of iodotyrosine deiodinase 1, the enzyme that aid in reuse of the hydrolyzed mono and diiodotyrosine and reenter pathways of hormone-producing in the thyrocytes (Yoshihara et al., 2019). Levothyroxine and fenugreek seeds extract have therapeutic effects against harmful effect of thiourea(Al-Zyadi, 2015).

The thyroid gland is entirely dependent on the pituitary hormone thyrotropin hormone (TSH) for the regulation of thyroid hormone production, TSH is the major stimulator of thyroid cell growth, differentiation and function (Kopp, 2001). There is a log-linear relationship between T3/T4 and TSH, and minor changes in T3/T4 lead to significant changes in TSH. T4 and T3 can then exert negative feedback on TSH levels (high levels of T3/T4 decrease TSH release from the anterior pituitary, while low levels of T3/T4 increase TSH release). T3 is the predominant inhibitor of TSH secretion(Pirahanchi et al., 2022). TSH levels increased in the present hypothyrodism rats model due to loss of negative feedback inhibition. Levothyroxine is a synthetic version of thyroxine. It replaces thyroxine and prevents the symptoms of hypothyroidism.

Thyroid sections revealed thyroid follicles of different sizes; their cavities contained acidophilic colloid with peripheral vacuolations. Thyroid follicles were lined by cubical follicular cells that exhibited rounded nuclei. Minute blood capillaries were extended between thyroid follicles, the follicular epithelium exhibited cubical cells. The thyroid gland is a unique endocrine gland; it is the largest, is superficially located and is specialized in the production, storage and release of the thyroid hormones thyroxine (T4) and triiodothyronine (T3)(Zaidi et al., 2004, Čakić-Milošević et al., 2004).

The thyroid gland activity is regulated by hypothalamic-pituitary-thyroid axis, including negative feedback loop. Insufficiency of the thyroid hormones in circulation stimulates pituitary to secrete thyroid-stimulating hormone (TSH), which has a critical role in the thyroid growth and activity (Zaidi et al., 2004). Under TSH stimulation, the thyroid gland undergoes enlargement, hyperplasia, neovascularization and morphological alterations of the thyrocytes related to their engagement in production. all these alterations were strongly expressed in hypothyroidism. It has been previously reported that thyroid hyperplasia induced by antithyroid drug such as methimazole was associated with the blood capillary enlargement and neovascularization (WOLLMAN et al., 1978, Patel et al., 1996).

The upregulation of TPO gene expression in primary hypothyrodism present rats model demonstrate a TSH-dependent increase of TPO mRNA and protein expression. High level of TSH in the present model of hypothyroidism binds to and activates the TSH receptor (TSH-R), The TSH-R is a G-protein coupled receptor located on the basolateral surface of thyroid follicular cells, with extracellular and three intracellular domains,

(Tuncel, 2017, Feldt-Rasmussen et al., 2021). This intracellular domain is important for coupling the TSH-R to G proteins, which can activate both adenylate cyclase and phospholipase C, generating the second messengers cAMP and phosphatidylinositol bisphosphate. However, studies performed primarily in mouse thyroid cells showed that TSHR regulates gene transcription at an intracellular site (Godbole et al., 2017). Dependent on internalization leading to nuclear localization of an activated transcription factors (Jang et al., 2022). rather than extracellular domain.

Hypothyroidism-associated ROS is the consequence of both increased production of free radicals and reduced capacity of the antioxidative defense and increased oxidative stress(Chakrabarti et al., 2016). Oxidative stress occurs when there is an imbalance between pro-oxidants and antioxidants, which occurs when oxidants cannot be neutralized through antioxidant defenses. Markers of oxidative stress associated with hypothyroidism have been studied in humans, showing a decrease in antioxidants and an increase in lipid peroxidation(Chainy and Sahoo, 2020). In hypothyroidism, thyroid follicles glutathione peroxidase activity (GPx1) as antioxidant biomarker was increased in dogs (Arostegui et al., 2023). the molecular mechanisms involved in regulating the expression and function of GPx-1, with an emphasis on the role of GPx-1 in modulating cellular oxidant stress and redox-mediated responses (Lubos et al., 2011). GPX1 can be regulated by transcriptional, post-transcriptional, and translational levels. Several investigators have shown that GPX1 expression is mainly regulated by several transcription factors and oxygen tension at the transcriptional level two oxygen-responsive elements (ORE) in the GPX1 promoter region. Oxygen-responsive element-binding protein (OREBP) can bind to them to regulate GPX1 transcription in response to oxygen tension (Stoytcheva and Berry, 2009). GPx1 gene expression can be explained by simultaneous metabolic processes, was influenced by cold temperature stress which resulted in a reduced metabolic rate (Do et al., 2019). Hypothyroidism-induced dysfunction of the mitochondrial respiratory chain can lead to the accelerated production of free radicals (Resch et al., 2002), glutathione peroxidase (GPx) family is the key enzyme for the detoxification of ROS in aquatic organisms(Do et al., 2019). While some authors suggest that tissues may be protected from oxidant damage by Levothyroxine improved oxidative status in patients with primary hypothyroidism (Masullo et al., 2018). Thyroid hormones elicit free radical generation and oxidative stress(Chainy and Sahoo, 2020).

Selenium has a U-shaped relationship with hypothyroidism disease, it bounds to more than 25 selenoprotein in thyroid gland cells, among them the three forms of deiodinas (Ventura et al., 2017) . The Selenium supplementation as a nanoparticles was effective in activation of these deiodinase enzymes and increase the metabolic reaction intracellularly leading to control the mechanism by which GPx was upregulated. Our results were also reported by Cheng et al. in renal cell carcinomas; they found that the increased levels of GPX1 were linked to lymph node metastases, advanced stage, and metastatic disease (Cheng et al., 2019).GPX1 expression is highly correlated with carcinogenesis and disease progression, GPX1 was highly expressed in various tumors, GPX1 overexpression was significantly correlated with the poor prognosis of L Besides, non-radiotherapy, chemotherapy, and low GPX1 expression were important factors affecting the better prognosis of LGG (Chen et al., 2022). According to Arczewska et al., GPX1 mRNA was increased in thyroid tumor tissue, but without reaching statistical significance. On the other hand, GPX1 mRNA level was increased in BRAFV600E mutated cancers (Arczewska et al., 2020).The low expression of selenium antioxidant molecules such as GPx1 and TrxR1 in papillary thyroid cancer cells suggested that oxidative stress was involved in thyroid malignancies, since free radicals were produced in excess (Metere et al., 2018).Selenoproteins play crucial roles in cellular processes such as DNA synthesis and protection from oxidative damage. Changes in the expression and activity of various selenoproteins in different types of thyroid cancer have been detected. Although selenium supplementation may be useful in the fight against thyroid cancer, the data so far are inconclusive.

Selenoproteins provide antioxidant protection for this tissue against the oxidative stress caused by free radicals and contribute, via iodothyronine deiodinases(Rua et al., 2023).

Treatment with levothyroxine has become the most common preparation used to treat hypothyroidism replacement a favorable side effect profile, ease of administration, good intestinal absorption, a long serum half-life, the stable T3 levels that are produced, and its low cost(Jonklaas et al., 2014). Using levothyroxine for primary hypothyroidism treatment associated with the TSH to be within lower end of reference range or suppressed below the reference range(Viswanath et al., 2007). Oral levothyroxine is FDA approved for treating primary hypothyroidism and pituitary thyrotropin suppression (Haugen et al., 2016, Chaker et al., 2017). As installed that normalizing of serum TSH in hypothyroidism patients is the purpose of treatment (Perros et al., 2021), the present used dose of levothyroxine, was adjusted to produce anormal level of TSH in compare with control group, This normalizing TSH restore the thyroid hormones (T3&T4) to normal level.

On the contrary supplementation of SeNPs showed no effective role on thyroid hormones and TSH in hypothyrodism .The non-elevated T3 and mild elevated T4 found by SeNPs supplementation is indicative that there were no clear role for the SeNPS in the synthesis of thyroid hormone, and can be contributed to fact that increased Se intake was negatively correlated with total T4 and the total T4/total T3 ratio (Liu et al., 2022), especially in males with adequate iodine intake. Se is particularly important for both thyroid homeostasis and the stability of the hypothalamic-pituitary-thyroid axis (Winther et al., 2020b, Gorini et al., 2021). The utilization and reserving functions of thyroidal thyroglobulin(TG) for Se is much lower than for iodine (Köhrle, 2023). The results of drug treatment were based on these facts as the TPO gene expression down regulated after treatment with the levothyroxin for a sufficient period enough to compensate for thyroid hormones and restore and normalize TSH. Levothyroxin alone or combined with SeNPs act in to two directions, first the replacement of thyroxine inhibits TSH synthesis and consequently reduced TPO gene expresion in thyroid follicles cells. TSH plays a pivotal role in the regulation of TPO gene expression in thyroid cells (Nazar et al., 2012). The TSH receptor (TSHR) is the major regulator of thyroid hormone biosynthesis in human thyrocytes by regulating the transcription of a number of genes including TG and TPO (Postiglione et al., 2002, Jang et al., 2022). Selenium work at the mRNA level in regulating hypothalamus -Pituitary- thyroid axis enzymes(Ma et al., 2021). Studies show that taking 200 mcg of selenium per day may help reduce antithyroid peroxidase (TPO) antibodies and improve well-being in people with Hashimoto's thyroiditis(Santos et al., 2018). Se is critical for the function of the thyroid, and it is particularly abundant in this gland. Supplementation is possible, but as Se has a narrow safety level, the risk of dangerous concentrations must be consider (Gorini et al., 2021), the benifet effects excerted by the SeNPS in methimazole hypothyrodism is attributed to dosage and adiminstration rout correctly chosen . Se is particularly important for both thyroid homeostasis and the stability of the hypothalamic-pituitary-thyroid axis, since all three deiodinase enzymes are selenoproteins (Rayman and Duntas, 2019). Iodide and selenium in thyroid gland make a system of antioxidant, and oxidant where as Selenoproteins are needed for triiodothyronine synthesis, its deactivation and iodine release, this system produces thyroid hormone and reactive iodine , and protect thyroid from hydrogen peroxide (Dijck-Brouwer et al., 2022). Our result showed the protective role of Se by regulating the expressions of GPx1 enzymes in thyroid gland cells, and in agree with documented role for the Se supplementation decreased the expression of GPx1 enzyme (Adeniran et al., 2022).

Conclusion: Evidantes from the present experiment has suggested that Se supplementation in the form of nanoparticles played role in regulating gene expressions of TPO and GPX1 with no evidence of increasing thyroid hormones.

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