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Original Article

Histological and Immunhistochemical Study on the Effects of Hypothyroidism on Seminal Vesicle of Adult Albino Rat and Possible Ameliorating Role of Selenium

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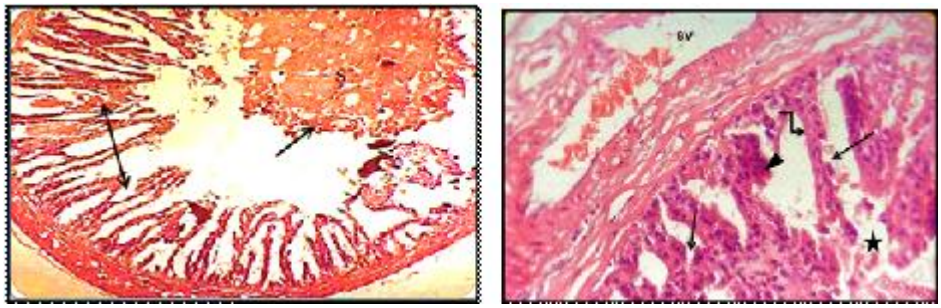
ABSTRACT

Hypothyroidism is a common metabolic disorder that influences the function of many organs including the seminal vesicle which plays a key role in male fertility process. This work aimed to evaluate the effects of selenium element on seminal vesicle of hypothyroid male rat. Forty male albino rats were used in this study, which were classified into 4 groups (10 animals in each group): Group I (control) received nothing. Group II received selenium in a dose of 10 µg/ b.w). Group III (hypothyroid) was given carbimazole in a dose of 6 mg/ kg b.w orally by gastric tube once daily for 3 months to induce hypothyroid state. In group IV (hypothyroid + selenium) the animals were treated by carbimazole as same as group III then selenium was added in last 2 months in a dose of 10 µg/ b.w. At the end of the experiment, blood samples were taken for the assessment of serum TSH, T4 and male hormone (testosterone), then animals were sacrificed, their seminal vesicle was removed and fixed in 10% formalin solution, and paraffin sections were prepared and stained by histological and immunhistochemical staining to be examined under the light microscope. Serum concentration of TSH significantly increased while the level of T4 and testosterone concentrations significantly decreased in groups III followed by group IV. Hypothyroidism caused reduction of secretory product, atrophy of mucosal folds and vacuolar degeneration of the secretary cells of seminal vesicle, while the immunhistochemical study showed low regulation of androgen receptor in the cells of this gland. However, the rats of group IV showed a significant improvement in hormonal level and histological structure of the seminal vesicle tissue compared with group II. So, this study indicated that selenium improved male fertility in hypothyroid state.

GRAPHICAL ABSTRACT



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Introduction

The seminal vesicles are pairs of accessory sex male glands, they secrete over two-third of seminal fluid, which has crucial effect on sperm viability, nutrition and stability of sperm chromatin [1]. Therefore, the normal function of this gland is imperative for fertility. The secretory activity of seminal vesicle depends on androgen level, which regulates the mitosis, morphogenesis of seminal vesicle epithelial cells and modulates the secretory of specific proteins [2] by its interaction with androgen receptors (ARs) [3].

Hypothyroidism is a metabolic disorder characterized by inadequate secretion of thyroid hormones (THs) [4]. Krassas et al indicated that thyroid dysfunction is attended with sexual dysfunction [5].

Selenium (Se) is a mineral that has an essential role in many biochemical and physiological processes including the biosynthesis of coenzyme Q [6] and activation glutathione peroxidase, which has antioxidant properties [7]. Messoudi *et al.* revealed that Se is an important antioxidant element that plays a crucial role in testicular development and function [8]. Liao *et al*. found that Se had antiperoxidative effect and capacity for inhibition of cisplatin hepatotoxicity in mice [9]. The purpose of the present study was to examine the effect of hypothyroidism on the histological structure of seminal vesicle gland and the possible protective role of Se.

Material and Methods

Carbimazole

It is an antithyroid drug obtained from the local markets in Iraq as 5 mg tablets supplied under the trade name NeoMercazole. It was dissolved in distilled water and orally administered to animals at a dose level of 6 mg/kg b.w [10].

Selenium

It is an antioxidant agent existing as sodium selenite powder. It was dissolved in distilled water and orally administered to animals at a dose level of 10 µg/kg b.w. [11].

Experimental protocol

Forty adult male Wistar albino rats weighing about (190- 225 g) and aged about three months were purchased from the animal house of veterinary college, University of Mosul. They were housed in polypropylene cages from the 1st of January till the 1st of April 2020. The animals were kept in well-ventilated cages, under standard environments, with free access to the standard diet and water. All animals had been examined carefully for general health status. The animals were divided into four groups (ten rats in each group) as follows: Group I (control group) received nothing. Group II rats were given distilled water daily for three months and Se was added orally by gastric tube once daily for the last two months. In group III (hypothyroid group) the animals in this group received carbimazole (6mg/b.w) dissolvent in water and given as 0.5ml orally by gastric tube once daily for three months to induce hypothyroidism. Group IV (hypothyroid + Se) rats were given carbimazole the same as group II but we added sodium selenite orally by gastric tube once daily for the last two months. Towards the end of the experiment, blood samples were collected from the retro-orbital venous plexuses. Then, all animals were killed by cervical decapitation, the seminal vesicle glands were dissected out, fixed in formalin (10%) solution, processed through a series of alcohol dilutions and embedded in the paraffin wax to get sections of 5µm thickness, stained with haematoxylin-eosin (H&E), masson trichome and immunhistochemical stain to be examined under the light microscope.

Hormonal assay

All blood samples were aspirated in the morning about 10-11 AM. The blood was centrifuged at 400 rpm for 15 mint then serum was separated and stored at −20°C. Serum thyroid-stimulating hormone (TSH), thyroxin (T4) and male hormone (testosterone) were determined by using the minividis technique.

Statistical analysis

All data were reported as mean±SE (standar error). The data were analyzed by Graphpad prism 8 using one-way ANOVA followed by the Student-Newman-Keuls multiple comparison tests with the level of significance set at $P \leq 0.05$.

Result and Discussion

The results of Group I (control) and Group II (Se treated) rats showed no significant difference; therefore, these two groups were collective in one group (control).

Hormonal Analysis

Hypothyroidism was established by measuring TSH and T4. The serum concentration of TSH, T4 level and testosterone are presented in Table 1 and Table 2, respectivly. Serum TSH significantly increased at ($p \leq 0.05$) while T4 significantly decreased in GII (hypothyroid) followed by GIV (hypothyroid + Se) in comparison to control group and there was a significant difference between both treated groups (GIII and GIV). The serum concentration of testosterone showed the least significant decrease in GII followed by GIV compared with control group. There was a significant differences between both treated groups.

Table 1: Serum concentration of thyroid hormones in different groups of male rats. Data are expressed as Mean±SE

Groups / Parameters	GI (Control)	GII (Hypothyroid)	GIV(Hypothyroid+Se)
TSH (µlU/ml)	2.68±190 A	5.86±0.03 B	4.42±0.11 C
T4 (nmol/L)	76.66±1.49 A	55.67±0.18 B	67.47±1.16 C

Different letters mean there is a significant difference at $p \leq 0.05$

Table 2: The serum concentration of testosterone in different groups of male rats. Data are expressed as Mean ± SE

Parameter	Control group	Hypothyroid group	Hypothyroid+Sel group
testosteroe (ng/ml)	4.39±0.37 A	0.68±0.10 B	2.34±0.17 C

Different letters mean there is a significant difference at $p \leq 0.05$

Histological Results

A-Haematoxyilin and Eosin stain

Control group (GI): The sections of seminal vesicle from this group showed irregular lumen filled with deeply stained eosinophilic secretion. The mucosa was folded into many primary and secondary fold which anastomosed with each other to form a honeycombed like ridges and depressions. The lining epithelium was made up of simple columnar cells rest on intact basement membrane and thin connective tissue lamina propria; below the mucosa there was a muscular layer (Figure 1 and 2).

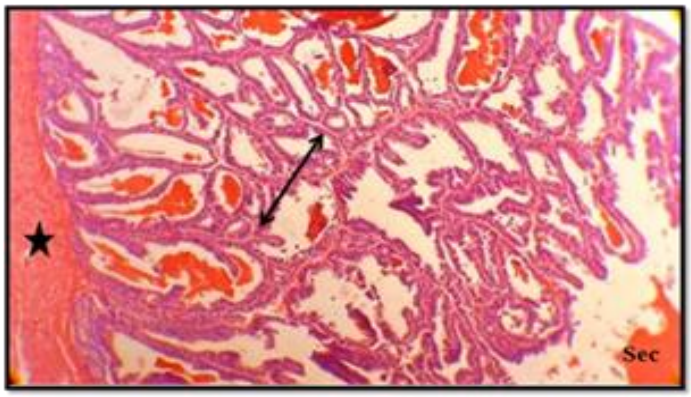

Figure 1: Microphotograph of seminal vesicle from control group, showing many mucosal folds (bihead arrow) surrounded by muscular layer (star) the lumen filled with secretion (Sec). H&E, 100X

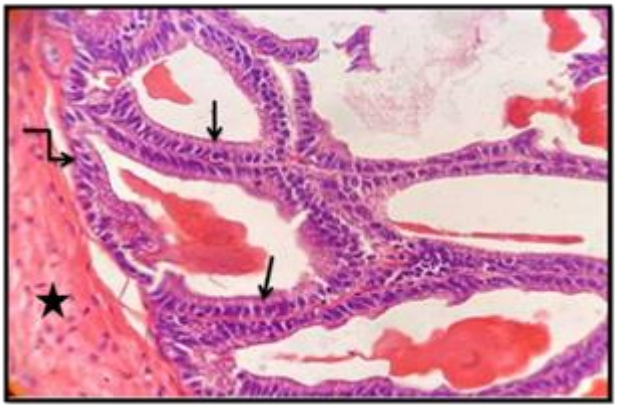

Figure 2: Microphotograph of seminal vesicle from control group, showing columnar epithelium (arrows) lining of aveoli rest on intact basement membrane (curved arrow) surrounded by muscular layer (star). H & E, 400X

Hypothyroid group (GIII)

The rats in this group were treated with carbimazole for three months; the sections of seminal vesicle showed atrophy and less folded mucosa with reduction of the secretion in the lumen compared to control group. Most of the epithelial cell lost its integrity, the epithelial cell height decreased at some places and stratification of epithelial cells was seen at other places. In addition, there was distruction and rupture of some secretory epithelium with desquamations of necrotic alveolar glandular epithelial in the lumen. The congested blood vesseles were observed with in the stroma of the gland (Figure 3 and 4).

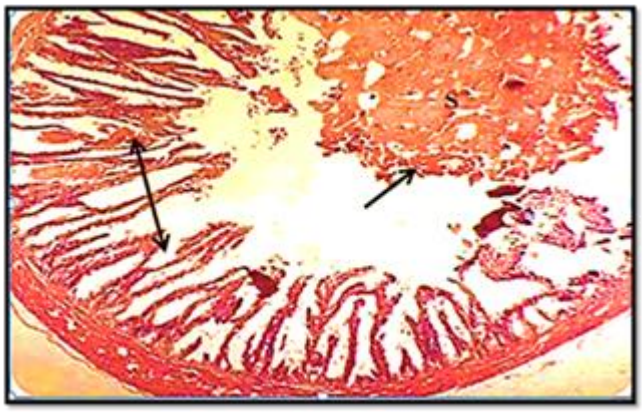

Figure 3: Microphotograph of seminal vesicle from hypothyroid group, showing atrophy of mucosal fold with reduction in their hight (bihead arrow) associated with reduced secretion (Sec). The lumen contains cellular debris of degenerated epithelium (arrow) H &E, 100X

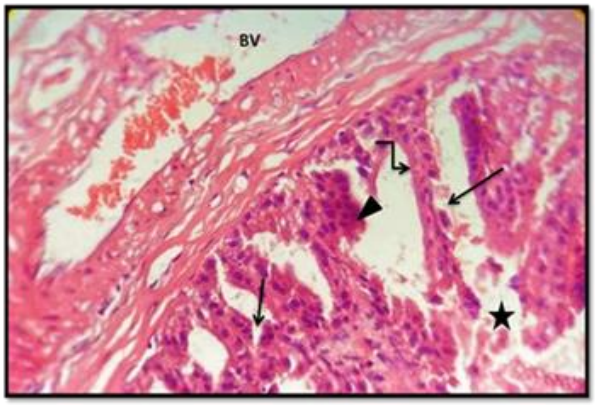

Figure 4: Microphotographof seminal vesicle from hypothyroid group, showing the epithelial cell height decreased at some places (curved arrow) and stratification of epithelial cells at other places(head arrow), distruction and rupture of some secretory epithelium (arrows) with desquamations of necrotic debries in the lumen(star), congested blood vessele the stroma (BV). H &E, 400X

Hypothyroid +Se treated group (GIV)

The sections from this group showed improvement in the mucosal fold with less atrophy in some fold. The lining epithelium of the alveoli showed variable degree of its height; it was either columnar, cuboidal or even sequamous. Also, there was few foci of multilayered epithelium compared with GII. Few dilated blood vesseles were observed in the stroma (Figure 5).

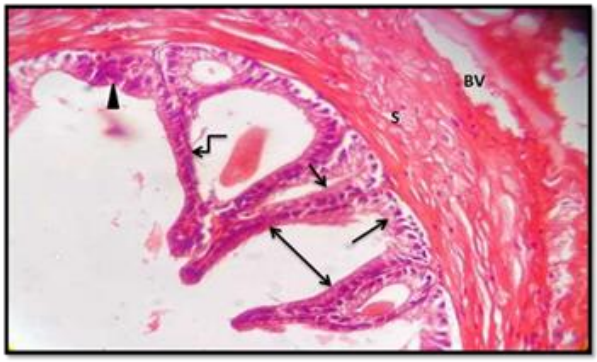

Figure 5: Microphotograph of seminal vesicle from hypothyroid treated with Se group, showing improvement of lining epithelium integrity, the epithelium of the alveoli showing variable degree of its height, either columnar (arrows), cuboidal (bihead arrow) or sequamous (curved arrow); also, there was foci of multilayered epithelium (head arrow). dilated blood vessele in the stroma (BV). H &E, 400X

B-Masson 's Trichome (MT) Stain

Control group

The MT stained sections from the seminal vesicle of control group demonstrated collagen fiber in the basement membrane and few fiber in interstitial stroma (Figure 6).

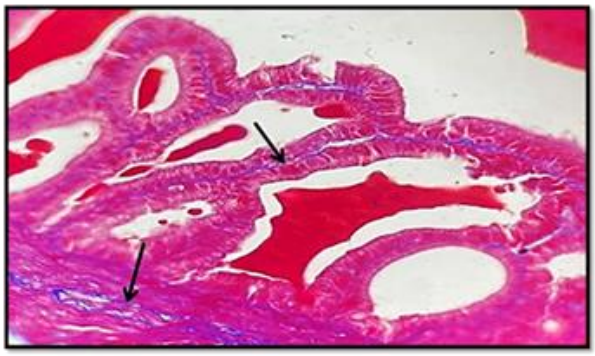

Figure 6: Microphotograph of a section in the seminal vesicle of control group showing normal thin distribution of collagen fiber in the basement membrane of lining epithelium (arrows) and in stroma .MT, X400

Hypothyroid group

The MT stained sections from the seminal vesicle of hypothyroid group demonstrated increased collagen fibers in the basement membrane and in interstitial stroma (Figure 7).

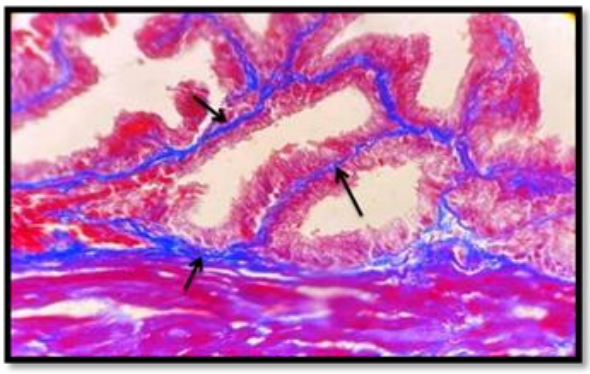

Figure 7: Microphotograph of seminal vesicle from hypothyroid group, showing thick collagen fibers in the basement membrane (arrows) and in the stroma (S) .MT, 400 X

Hypothyroid treated with selenium group

The seminal vesicle from this group demonstrated increased collagen fibers in the basement membrane and in interstitial stroma but less than that observed in hypothyroid group (Figure 8).

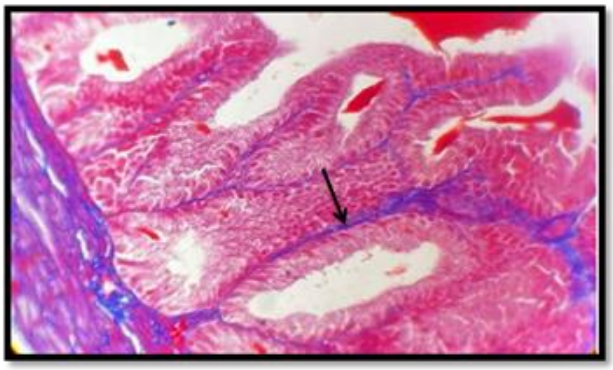

Figure 8: Microphotograph of seminal vesicle from hypothyroid treated with Se group, showing mild to moderate thickness of the collagen fibers in the basement membrane (arrow) and in stroma (S). MT, 400 X

Immunhistochemical study

Control group

The seminal vesicle sections showed AR expression in the secretory epithelium while in stromal area, the AR expression was observed in the nuclei of smooth muscles and stromal cells (Figure 9).

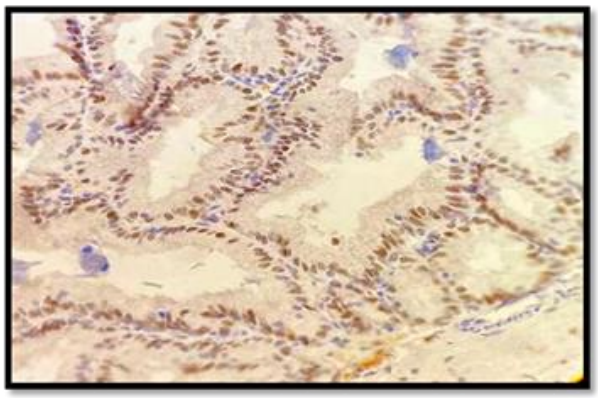

Figure 9: Microphotograph of seminal vesicle from control group, showing AR expression in the secretory epitheium (arrows) and nuclei of stromal cell (curved arrow). IHC, 400X

Hypothyroid and Hypothyroid with Se groups

Induction of hypothyroidism in male rats suppressed the expression of AR in the seminal vesicle compared with control group (Figure 10), while the co-administration of Se increased AR expression in the glands compared to hypothyroid group (Figure 11).

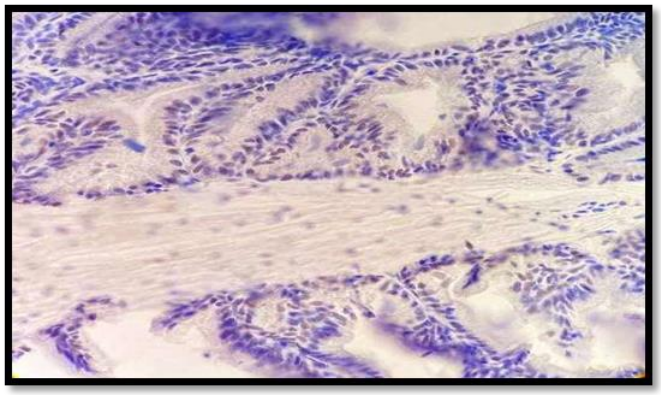

Figure 10: Microphotograph of seminal vesicle from hypothyroid group, showing weak AR expression in secretory epithelium (arrows). IHC, 400X

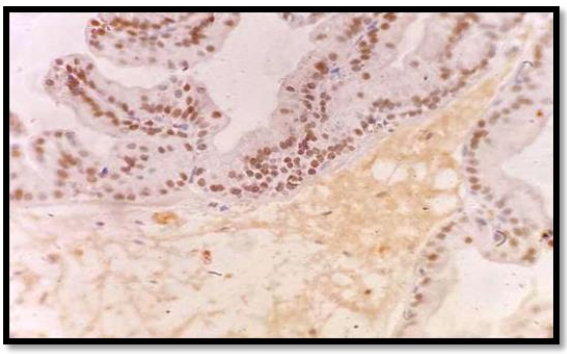

Figure 11: Microphotograph of seminal vesicle from hypothyroid treated with Se group, showing +ve AR expression in secretory epithelium (arrows). IHC, 400X

Hypothyroidism is a clinical condition caused by reduction in the secretion of THs from thyroid gland. It adversely affects the function of many organs including the reproductive organs [12]. A previous study has confirmed the role of thyroid hormones in testicular development [13], while another study has suggested that thyroid dysfunction in adult men is related to an abnormality in sexual activity and impaired fertility [14].

TSH and T4 were used as an indicator for normal thyroid function in human and other animals [12]. The induction of hypothyroidism in our study was confirmed by significant reduction in T4 and elevation of TSH concentration compared to control group. This result is in line with another past study that used carbimazole for six weeks to induce hypothyroidism in experimental animales [15]. The reduction of T4 level may be attributed to the action of carbimazole that inhibits iodination of tyrosin in the thyroid gland; as a consequence, the concentration of TSH was elevated by positive feed-back effect on hypophysis [16]. However, the thyroid function was improved by Se given to hypothyroid rats in which the level of T4 increased and TSH decreased compared with hypothyroid groups. The result of the current work is in accordance with that of another researchers , who found an improvement in thyroid function after addition of Se to hypo male rats [17]. The improvement of thyroid activity observed in our study may be attributed to the role of Se in thyroid homeostasis. Se is regarded as a part of selenoproteins, which have a antioxidant activity to protect the thyroid gland from the harmful effects of free radicles generated during the thyroid function. In addition, Se incorporates to iodothyronine deiodinase enzyme, which has an important role in the convertion of T4 to more active T3 and in THs metabolism [17].

Moreover, the serum concentration of testosterone level in male rats significantly reduced in hypothyroid group; the current results are in agreement with those observed by another researcher [18]. Liu *et al*., reported that hypothyroidism is associated with suppression of cytochrome enzyme expression and as a consequence inhibits the synthesis of androgen [18]. Likewise, Selva and Hammond attributed the reduction of sex hormone level to the suppressive effect of hypothyroidism on the production of sex hormone binding globulin (SHBG) from the liver [19]. The co-administration of Se in the current study to hypothyroid rats resulted in a significant elevation of testosterone level. El-Maraghy and Nassar stated that Se had stimulatory effect on testosterone synthesis and improved fertility in male [20]. The action of Se may be attributed to its antioxidant effect that protects the leydig cells in male from oxidative damage [21].

Histological study in the present work revealed many changes in the seminal vesicles structures of hypothyroid rats. The alveoli showed area of epithelial hyperplasia, degenerative changes of lining epithelium and atrophy of mucosal fold with reduction of their height. The MT stained sections demonstrated increase in the collagen fibers content of the gland in this group compared with the control group. Lotti1 *et al*., stated that THs had a positive effect on the size and function of seminal vesicle and suggested a probable remolding action of these hormones on

the seminal vesicle structures [22]. Another study demonstrated that both the prostate and seminal vesicle were androgen dependent glands [15]. Testosterone has an important role in the preservation of the mucosal fold and smooth muscle in the seminal vesicle [23]. In the present study the increased amount of collagen fibers in the stroma of the seminal vesicle might have happened due to the decline of testosterone level this confirmed by observation of increased stromal thickening of prostate with age in whome the androgen level were low[24]. Moreover, tissues hypoxia occurred due to hypothyroidism that may have promoted the formation of collagen fibers [25]. Saber *et al*., attributed the histopathological changes in prostate of hypothyroid male rats to the effect of free radicles and increased lipid peroxidation induced by hypothyroidism [15].

THs may influence the activity of the reproductive organs via its effect on the receptors of the steroid hormones [26]. In the current study, induction of hypothyroidism led to down regulation of AR in the secretory cells of the gland. Issa and El-Sherif found that subclinical hypothyroidism in male rats caused low regulation of ARs in cells of the testis [27]. This result may be attributed to the effect of thyroid dysfunction on the sex hormones level since this hormone regulates the expression of steroid receptors [28].

Concomitant use of Se along with carbimazole in the our study improved the effect on the histopathological findings that were already observed in hypothyroid group. Many studies were performed to evaluate the protective effect of Se against tumors, diseases and toxicity of different agents. Hoenjet *et al.*, showed that Se could be used in the inhibition of prostatic cancer [29]. Lance *et al*., confirmed the effect of Se on colonic cancer[30]. Seema *et al*., pointed to the protective role of Se against testicular toxicity induced in rat by nicotin [31]. This ameliorative effect of Se may be attributed to its antioxidant activity and to its role in protection of cell damage produced by increased free radicle [15].

Conclusion

In conclusion, the current study reported the enhanced effect of Se on structural changes of seminal vesicle induced by hypothyroidism and hence improved male fertility in hypothyroid state.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The authors assert no conflict of interests of the manuscript.

References

[1]. Suja K.P., Jayalekshmy A., Arumughan C., *J. Sci. Food Agric.*, 2005, **85**:1779 [Crossref], [Google Scholar], [Publisher]

[2]. Basiri M., Asadi-Shekaari M., Ezzatabdipour M., Sarv Azad A., Nematollahimahani S.N., *Cell J.*, 2016: **18**:458 [Crossref], [Google Scholar], [Publisher]

[3]. Bennett N.C., Gardiner R.A., Hooper J.D., Johnson D.W., Gobe G.C., *Int. J. Biochem. Cell Biol.*, 2010, **42**:813 [Crossref], [Google Scholar], [Publisher]

[4]. Mullur R., Liu Y.Y., Brent G.A., *Physiol Rev.*, 2014, **94**:355 [Crossref], [Google Scholar], [Publisher]

[5]. Krassas G.E., Poppe K., Glinoer D., *Endocr Rev.*, 2010, **31**:702 [Crossref], [Google Scholar], [Publisher]

[6]. Mangiapane E., Pessione A., Pessione E., *Curr Protein Pept. Sci.*, 2014, **15**:598 [Crossref], [Google Scholar], [Publisher]

[7]. Boitani C., Puglisi R., *Adv. Exp. Med. Biol.*, 2008, **636**:65 [Crossref], [Google Scholar], [Publisher]
[8]. Messoudi I., Banni M., Said L., Said K., Kerkeni A., *Chem. Bio. Interact.,* 2010, **188**:99 [Crossref], [Google Scholar], [Publisher]
[9]. Liao Y., Lu X., Lu C., Li G., Jin Y., Tang H., *Pharmacol. Res.*, 2008, **57**:125 [Crossref], [Google Scholar], [Publisher]
[10]. Zaidi T.M., Khan A.A, Hasan B.M. and Faruqi A.N., J. Anat. Soc. India., 2004, **53:**14 [Google Scholar]
[11]. Swathy S.S., Panicker S., Indira M., *Ind. J. Physiol. Pharmacol.*, 2006, **50**:215 [Google Scholar]
[12]. Krassas G.E., Markou K.B., *Hormones*, 2019, 18:365 [Crossref], [Google Scholar], [Publisher]
[13]. Carani C., Isidori A.M., Granata A., Carosa E., Maggi M., Lenzi A., *J. Clin. Endocrinol. Metab.*, 2005, **90**:6472 [Crossref], [Google Scholar], [Publisher]
[14]. La Vignera S., Vita R., *Int. J. Immunopathol Pharmacol.*, 2018, **32**:1 [Crossref], [Google Scholar], [Publisher]
[15]. [32]. Saber A.S., Hoda A.M., Amany E.N., *Am. J. M. Sci.*, 2012, **2**:5 [Crossref], [Google Scholar], [Publisher]
[16]. Abraham P., Avenell A., Park C.M., Watson W.A., Bevan J.S., *Eur. J. Endocrinol.*, 2005, **153**:484 [Crossref], [Google Scholar], [Publisher]
[17]. Ventura M., Melo M., Carrilho F., *Int. J. Endocrinol.*, 2017, **2017**:1297658. [Crossref], [Google Scholar], [Publisher]
[18]. Liu, Guo, Hu, Weng, Tian, Xu, Heng, Liu, Ding, Yang and Zhang. *Chin. J. Physiol*, 2018, **61**:152 [Crossref], [Google Scholar], [Publisher]
[19]. Selva D.M., Hammond G.L., *J. Mol. Endocrinol.*, 2009, **43**:19 [Crossref], [Google Scholar], [Publisher]
[20]. El-Maraghy S.A., Nassar N.N., *J. Biochem. Mol. Toxicol.*, 2011, **25**:15 [Crossref], [Google Scholar], [Publisher]
[21]. Qazi I.H., Angel C., Yang H., Pan B., Zoidis E., Zeng C.J., Han H., Zhou G.B., *Molecules.*, 2018, **23**:3053 [Crossref], [Google Scholar], [Publisher]
[22]. Lotti F., Maseroli E., Fralassi N., Degl'Innocenti S., Boni L., Baldi E., Maggi M., *Hum. Reprod.*, 2016, **31**:518 [Crossref], [Google Scholar], [Publisher]
[23]. Welsh M., Moffat L., Jack L., McNeilly A., Brownstein D., Saunders P.T., Sharpe R.M., Smith, L.B., *Endocrinology,* 2010, **151**:3374 [Crossref], [Google Scholar], [Publisher]
[24]. Fledman H.A., Longcope C., Derby C.A., Johannes C.B., Araujo A.B., Covello A.D., Bremner W.J., McKinlay J.B., *J. Clin. Endocinol. Metab.*, 2002, **87**:589 [Crossref], [Google Scholar], [Publisher]
[25]. Hofbauer K.H., Gess B., Lohaus C., Meyer H.E., Katschinski D., Kurtz A., *Eur. J. Biochem.*, 2003, **270**:4515 [Crossref], [Google Scholar], [Publisher]
[26]. Krassas G.E., Poppe K., Glinoer D., *Endocr Rev.*, 2010, **31**:702 [Crossref], [Google Scholar], [Publisher]
[27]. Issa N.M., El-Sherif N.M., *Austin J. Anat.*, 2017, **4**:1065 [Google Scholar], [Publisher]
[28]. Arambepola N.K., Bunick D., Cooke P.S., *J. Endocrinol.*, 1998, **156**:43 [Crossref], [Google Scholar], [Publisher]
[29]. Hoenjet K., Dagnelie P.C., Delaere K.P.J., Wijckmans N.E.G., Zambon J.V., Oosterhof G.O.N., *Eur. Urol.*, 2005, **47**:433 [Crossref], [Google Scholar], [Publisher]
[30]. Lance P., Alberts D.S., Thompson P.A., Fales, L., Wang F., Jose J., Jacobs E.T., Gooman P.J., Darke A.K., Yee M., Minasian L., *Cancer Prev. Res. (Phila).*, 2017, **10**:45 [Crossref], [Google Scholar], [Publisher]
[31]. Seema, P., Swathy S.S., Indira M., *Biol. Trace Elem. Res.,* 2007, **120**:212 [Crossref], [Google Scholar], [Publisher]

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