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Hypothyroidism: morphological and metabolic changes in the testis of adult albino rat and the amelioration by alpha lipoic acid

Running title: Hypothyroidism, alpha lipoic acid, and testis

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Abstract

The objective of this study is to evaluate the influence of carbimazole-induced hypothyroidism on testis of adult albino rats and the probable protective effect of α -lipoic acid (ALA). The rats were divided into four groups; Control, α -lipoic acid group, carbimazole, and carbimazole+ α -lipoic acid groups. Rats were exposed to ALA (60 mg/kg BW) or carbimazole (1.35 mg/kg BW) or both via gavages for 30 days. Morphometric analysis revealed a significant decrease in tubular diameter, germinal epithelium thickness, and interstitial space as compared to the controls. Also, rats exposed to carbimazole showed a significant decline in testicular weight, sperm motility, and count. Also, deterioration of the testicular architecture. ALA supplementation resulted in a significant improvement in the tubular diameter and germinal epithelium thickness, but no significant improvement regarding interstitial space was observed. Another observation was the significant decline in serum testosterone and FSH in the carbimazole group, indicating reduced

steroidogenesis. A significant reduction in reduced glutathione content was detected in the testes of the carbimazole group over the controls, while malonaldehyde (MDA) concentration significantly increased. Conversely, ALA supplementation ameliorated the toxicity induced by hypothyroidism as illustrated by enhanced reproductive organ weights, testosterone, LH, and FSH levels, testicular steroidogenesis, and oxidative stress parameters. Hypothyroidism altered testicular antioxidant balance and negatively affected spermatogenesis. On the other hand, ALA through its antioxidant properties alleviated testicular toxicity in carbimazole-exposed rats.

Key words: alpha-lipoic acid, carbimazole, hypothyroidism, testis, rat

INTRODUCTION

In the past decades, clinical studies revealed that the thyroid hormone plays a mandatory role in spermatogenesis and steroidogenesis. It is now known that T3 regulates the testicular growth and maturation, in different mammals [27]. The efficiency of spermatogenesis is directly correlated to the number of functional Sertoli cells entrenched during adulthood [40]. Thyroid hormones have a well-known physiological role in modulating the process of oxidative stress caused by reactive oxygen species (ROS) [28]. Its deficiency is known to induce hypothyroidism and oxidative stress and perhaps might lead to testicular dysfunction and infertility [20]. In males, hypothyroidism is accompanied by hypogonadism and other abnormalities, like a declined serum testosterone [24]. Furthermore, hypothyroidism can alter sperm morphology and motility [23]. Carbimazole mode of action is through its active form Methimazole (1-methyl-2-mercaptoimidazole), which inhibits the iodination of tyrosine catalyzed by thyroid peroxidase inducing lowering of circulating thyroid hormones [31]. In this regard, carbimazole has been used to induce experimental hypothyroidism [6]. α -lipoic acid (ALA), is one of the cofactors for multi-enzyme complexes in mitochondria, enhances the uptake of glucose by the cells, and modulates the activity of various signaling molecules and transcription factors [41]. Moreover, ALA has a lipophilic character, it is can cross cell membranes, with dihyrolipoic acid (DHLA) it can overcome free radicals efficiently in both lipid and watery phases [16]. Various reports in the literature suggested that ALA can improve body resistance to free radicals [42]. Also, ALA can recycle other important antioxidants, including glutathione and vitamin C [21]. To date, less attention has been directed towards identifying the precise role of ALA in ameliorating the toxic effects caused by oxidative stress, especially in the hypothyroid state. From this perspective, we designed this study to determine the effects of

carbimazole-induced hypothyroidism on testicular structure and function and the possible ameliorating role of ALA supplementation.

MATERIALS AND METHODS

Animal models and groups treatment

Forty healthy adult males (21 weeks old and weighed 200 to 250g) albino rats of Wister strain were obtained from Zagazig Vet. Medicine animal house, Egypt. All groups of rats were kept *ad libitum* and were grouped (n = 5 per cage) in standard special cages made from plastic. Before experimentation, all animals were accommodated for two weeks at [Temperature: 24-26 °C; and in a 12 h light/12 h dark cycle]. Rats then divided into four main groups (10/ group). Group 1 (Control) received 2 ml of distilled water with 2 ml of olive oil orally. Group 2 (α -lipoic acid group) administered 2 ml of distilled water plus ALA (60 mg/kg BW) [11] dissolved in 2 ml of olive oil. Group 3 (carbimazole group) administered 2 ml of olive oil plus carbimazole (1.35 mg/kg BW) [32] dissolved in 2 ml of distilled water. Group 4 (carbimazole + α -lipoic acid group) administered ALA (60 mg/kg BW) dissolved in 2 ml of olive oil and carbimazole (1.35 mg/kg BW) dissolved in 2 ml distilled water. Each rat daily administered a fresh single oral dose via gavages for 30 days. The Guide for the Care and Use of Laboratory Animals, 8th edition was our guide during all experimental processes [1]. The Institutional Animal Care and Use Committee at Zagazig University (ZU-IACUC/3/F/29/2018) approved the present study. All chemicals were purchased from Sigma Chemicals Company (St. Louis, MO).

Blood collection

The rats were sacrificed under ethyl ether anesthesia after 24 hours of the last dose [13]. Blood samples were obtained by cardiac puncture and then centrifuged at 4000 rpm for a quarter of an hour after allowing the blood to clot for two hours at room temperature. The sera from all rats were kept at -20 °C till the proceeding of the biochemical determinations. We take in mind, to avoid the repeated freezing and thawing of preserved samples.

Extraction and preparation of gonads

The testes were removed from all groups by laparotomy and the epididymis was obtained, removed, and homogenized at 37 °C in 2 ml of Hank's buffer solution. Then caudal epididymis sperms numbers were detected by hemocytometry technique [37].

The right testes were weighed and washed in cold physiologic saline and dried with filter paper. The volume was estimated, then they were fixed in 10% buffered formaldehyde solution, and then processed and embedded in paraffin wax. The left testes from all animals were weighed and their volume was estimated and recorded. Testes of all groups were homogenized in 50 mM phosphate buffer (pH 7) containing 0.1 mM EDTA to give 10% homogenate (w/v). The testicular homogenates were centrifuged for 10 mins at 1000 rpm and the supernatant was separated and then after used for oxidative stress evaluation [37].

Light microscopic analysis

Sections from the testis at 4-5 μm thick were obtained and stained with eosin and hematoxylin to assess general histological architecture [3]. Thirty different testicular sections from 10 different rats in each group were processed for immunohistochemistry [39]. In brief, 3% H₂O₂ in PBS was used to block the endogenous peroxidase activity. Immersing the slides in citrate sodium solution (10 mM, pH 6.0) for 15 min at 95 °C was done for antigenic retrieval. The sections were kept in diluted primary antibodies overnight at 4 °C; (Myoid cell marker) Rabbit polyclonal anti- α -SMA “smooth muscle actin” (diluted 1:3000; Catalog No. ab5694, Abcam, Cambridge, UK), (Androgen receptor antibody) mouse anti-“androgen receptor” (N-20, sc-816; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) diluted 1:100, and mice anti-Vimentin as (Sertoli cell marker, 1:50, Santa Cruz, CA, USA). After addition of avidinbiotin complex, Mayer’s hematoxylin was used as a counterstain. All sections were photographed and examined using a photomicroscope (Leica DM500, German).

Morphometry

ImageJ software was used for image analysis and morphometry according to Wayne Rasband-National Institute of Mental Health (Bethesda, Maryland, USA). Seminiferous tubule diameter, germinal epithelium thickness, and interstitial space thickness (μm) were measured in six sections per testis from ten different rats in each group at magnification power X400. Also, numbers of Sertoli cells and Leydig cells were also counted in the same slides. In addition, the area percentages of androgen receptor, α smooth muscle actin, and vimentin expression were assessed.

Biochemical analysis

Blood samples from the ten different rats in each group were used to determine the hormone levels in the serum. Serum levels of triiodothyronine (T3) (mouse/rat ELISA Kit) (Abnova, Taiwan), and thyroxin (T4) (Rat ELISA Kit) (MyBioSource, Inc. USA), were determined by

colorimetric method according to the manufacturer instructions. rTSH kit – Biotrak Assay System was used to determine TSH levels. The sensitivity of the rTSH assay was 0.05 ng/tube, with cross-reactivity 100% with rat TSH and 15% non-specific binding. The procedures were performed following the specifications of the manufacturer's manual. FSH level was estimated using rat follicle-Stimulating Hormone (FSH) ELISA Kit, Novus Biologicals (KA2330) by Colorimetric method [33]. LH level was estimated using rat luteinizing hormone (LH) ELISA kit, Novus Biologicals (NBP2-61257) by colorimetric method range from 1.2 to 280 mIU/m according to the manufacturer instructions. Testosterone level was estimated using rat testosterone ELISA Kit, Aviva Systems Biology (OKCA00179) by a colorimetric method (OD450nm) [4]. Malondialdehyde (MDA) level was assessed by incubating the testis homogenate at 100°C in one mL of trichloroacetic acid (10%) and one mL of thiobarbituric acid (0.67%) for a half of an hour. The amount of MDA concentration was expressed by the colored complex obtained at 535 nm absorbance [30]. Reduced glutathione (GSH) content in the tissue homogenate was determined, where a mixture formed from 250µL of testicular homogenate (10%), 250 µL distilled water, 50µL of TCA (50%). The mixture was shaken for 15 min and centrifuged for 10 mins at 3000 rpm. The 10µL of supernatant was mixed with 400µL Tris buffer (0.4 M) and (pH= 8.9) and 10 µL of 5,5-dithio-bis-2 nitrobenzoic acid (DTNB). The obtained color complex was detected by a spectrophotometer at 512 nm [38].

Statistics

The obtained data of our results were statistically analyzed by Graph Pad Prism 5.01 (GraphPad Software, San Diego, CA, USA). Quantitative data were tabulated as mean and SD when normally distributed or median and interquartile range if not normally distributed. Analysis of variance (ANOVA) used to test mean values differences for all experimental groups and Bonferroni's multiple comparison test was used as a post hoc test. The statistical significance of the results was considered at (P-value < 0.05).

RESULTS

Hypothyroidism was induced by administering carbimazole (1.35 mg/kg BW). This resulted in a decrease in serum concentrations of total T3, and T4. Also, this was accompanied by a significant increase in the serum TSH levels. Moreover, ALA supplementation did not affect the hypothyroid state in the carbimazole + α -lipoic acid group (Figure 1A). A decrease in testicular weight and volume of hypothyroid rats was observed (Figure 1B).

Gross measurements and semen analysis

The average testicular sperm count was significantly reduced in the carbimazole group when compared to the control group. Supplementation of ALA significantly restored the sperm count in the testis of the carbimazole + α -lipoic acid group (Figure 1C). The mean sperm motility in the carbimazole group was significantly low when compared to the Control group. However, a significant elevation was detected in the carbimazole + ALA group after ALA supplementation.

Light microscopic analysis

In the control group, the testicular sections expressed normal architecture with average sperm density (Figure 2A and B). However, in the carbimazole group, there was a deposition of homogenous substance in the interstitial spaces, which was associated with congestion and disturbance in the tubular contour. Also, destruction in some tubular walls was noted. Besides, testicular sections expressed a remarkable detachment of germinal epithelium with decreased spermatocytes density. Furthermore, vacuolations in the interstitial space and in between spermatocytes, and scarce Sertoli cells with scattering and stripping off spermatids were observed and Leydig cells nearly disappeared (Figure 2C and D). Moreover, the tubular diameter, germinal epithelium thickness, and interstitial space were decreased when compared to the control group. On the other hand, the supplementation of ALA resulted in a significant improvement in the tubular diameter and germinal epithelium thickness. However, no significant improvement regarding interstitial space was observed (Table 1). In the carbimazole + α -lipoic acid group, the integrity of germinal epithelium was maintained with minute areas of detachment and Leydig cell clusters were preserved. Besides, refinement in spermatocyte density was noted. However, no deposition of homogenous substance and the regularity and maintenance of the tubular contour were not observed. Also, spermatids appeared scattered (Figure 2E and F). Sertoli cell and Leydig cell numbers were significantly decreased in the carbimazole group when compared to the control. Moreover, their numbers were significantly increased more than the carbimazole group after ALA combination (Table 1).

Immunohistochemistry

In the testes of the Control group, androgen receptor expression was prominent in interstitial cells of Leydig and the peritubular myoid cells, but it was weak in the Sertoli cells in all stages of spermatogenesis. In contrast, the androgen receptor expression was limited to certain interstitial cells with a decrease in the area percent in the carbimazole group. The decrease in androgen receptor expression in Sertoli cells was prominent in all stages of spermatogenesis especially stage

IV-VIII. Interestingly, the group that received carbimazole + α -lipoic acid showed maintenance of the androgen receptor expression in Leydig cells and some peritubular myoid cells with improvement in the area percentage (Table 1 and Figure 3 A, B and C). Regarding α smooth muscle actin expression, it was limited to the peritubular myoid cells and it appeared as a very thin bilaminar line with very potential space in between laminae, and in the wall of the interstitial blood vessels in the Control group. In contrast, α smooth muscle actin expression in the carbimazole group showed areas of thickening and fusion between the laminae. Besides, it was associated with prominent thickening in the wall of blood vessels, which in turn increased the surface area percentage. However, the testes of the carbimazole + α -lipoic acid group displayed maintenance of the control expression pattern (Table 1 and Figure 3 D, E, and F). We also analyzed the immunocontent of vimentin expression. The results showed that vimentin was prominently expressed in the cytoplasm of the Sertoli cells with clear continuity between both apical and basal segments and the interstitial tissue in the Control group. On the contrary, Sertoli cells' destruction was elucidated by the pattern of vimentin expression in the carbimazole group. There was a destruction of the apical segment of Sertoli cells with the scarce appearance of the basal segment in most of the tubules. Besides, there was a lack of expression in certain areas of the interstitial tissue which appeared empty of cells. This resulted in a decrease in the area percentage of vimentin expression. On the other hand, limited conservation of the expression pattern of the control in some Sertoli cells was detected in the carbimazole + ALA group. Also, the interstitial tissue was partially protected showing fractional improvement in area percentage of vimentin expression. (Table 1 and Figure 4 A, B and C)

Biochemical analysis

Serum levels of testosterone were significantly decreased in the carbimazole group compared to the control group. Also, FSH concentrations showed a significant decline in the carbimazole group when compared to the control group. While the level of LH showed a significant increase in the carbimazole group compared to the control group. The results were improved after ALA administration in the carbimazole + ALA group, with a significant increase in testosterone and FSH levels (Figure 5(A)). GSH contents in the carbimazole group were showed a significant decline when compared to the control group. (Figure 5(B)). A comparison of the MDA contents of testis showed a significant increase in the carbimazole group when compared to the control group. Interestingly, the results improved in the carbimazole + ALA group, and a significant decrease was observed in MDA (Figure 5(B)).

DISCUSSION

The impact of thyroid diseases on male reproduction has been an area of controversy for many years ago, due to the reports of the inability of the testis to respond metabolically to thyroid hormones [15]. Yet, in the last decades, some clinical trials have concluded that thyroid hormones have a great role in the process of testicular spermatogenesis even at and after puberty [14]. However, some authors suggested that hypothyroidism does not a matter influence the functions or the size of the testis or seminiferous tubules [10]. Thyroid hormones are considered to be one of the main factors in cell metabolism regulation and thus play a significant role in oxidative stress [8]. The impetus of the present study is to determine the ameliorating role of ALA supplementation on the damage caused by carbimazole-induced hypothyroidism on testicular tissue of the experimental animal model. The obtained data of the present study approved that carbimazole is can decrease the testicular volume and tubular diameter, germinal epithelium thickness, sperm count as compared to the control group. Besides, congestion and disturbance in tubular contour with damage to some tubular wall and very limited expression of androgen receptor to certain interstitial cells were observed. Moreover, detachment of germinal epithelium decreased spermatocytes density, and destruction in Sertoli cells was recorded. The above-obtained data came in accordance with Prathima et al. [32] who reported that, when rats exposed to carbimazole showed major histological deterioration such as congestion of blood vessels, hemorrhage, and destruction of interstitial cells with degeneration of spermatogenic cells with the irregular cell membrane. The report of Yousofvand et al. [43] revealed that hypothyroidism induced by a chemical called propylthiouracil (PTU) was able to induce a significant reduction in testes weight [43]. In another study, carbimazole at a dose of (1.35 mg/kg) for three or six weeks resulted in the disorganization of germinal cells, a decrease thee diameters of the seminiferous tubules and the height of testicular tissue epithelium. This was associated with congestion of interstitial blood vessels and edema [35]. This may be related to the increased blood endotoxins and the state of oxidative stress that lead to capillary destruction, exudation and apoptosis. This is in accordance with Jalilvand et al. [19] that hypothyroidism increases TUNEL positive cells in the testis. Examination of the testes of rats treated with carbimazole and ALA showed improvement in tubular diameter and germinal epithelium thickness, and no change was seen in interstitial space regularity. Also, maintenance of tubular contour with nearly normal expression of androgen receptors, vimentin, in alpha-smooth muscle actin was observed. These data inconsistency with a previous result [5], which revealed that ALA works at two levels intracellular and extracellular (at cell membrane level). Thus, giving double protection and improving the pathology of testicular tissue with normal expression of different immune markers. Jewish [11] concluded that the ALA has an ameliorative role against

toxicity induced by hypothyroidism. Also, Prathima et al. [32] reported that ALA acts as a potent antioxidant and can reverse the histological changes in the testicular tissue of adult rats induced by carbimazole. Androgen receptor expression in Sertoli cells was reduced due to the state of oxidative stress induced by admission of ethanol [17]. This agreed with the finding of this experiment in which Androgen receptor expression was limited to certain interstitial cells with a decrease in the area percent in the carbimazole group. The decrease in androgen receptor expression in Sertoli cells was prominent in all stages of spermatogenesis especially stage IV-VIII. As reported by Horibe et al. [18] that marked decrease in Androgen receptor expression was evident in all stages of the spermatogenic cycle in the testes of rats treated with ethanol. Reduction in Androgen receptor expression can affect the pattern of α smooth muscle actin expression [29]. In the current study α smooth muscle actin expression showed prominent thickening in the wall of blood vessels, which in turn increased the surface area percentage after Carbimazole supplementation. On the other hand O'Hara et al. [29] showed reduction in α smooth muscle actin expression following Androgen receptor expression in transgenic mice. Also Gosteli-Peter et al. [12] mentioned that T3 increased dramatically α smooth muscle actin mRNA and protein accumulation. Vimentin can be considered as Sertoli cell marker. It is dependent on spermatocytes renewal and attachment to Sertoli cells which is altered by oxidative stress induced by busulphan [22]. This goes with the pattern noticed in this experiment regarding vimentin expression in the control group. As well as, the destruction in the apical segment of Sertoli cells with the scarce appearance of the basal segment in most of the tubule after carbimazole treatment. On the contrary, Zamoner et al. [44] mentioned that no change in vimentin expression by RT-qPCR in the testis of hypothyroid rats however increase in its phosphorylation and its content in the cytoskeleton of the testis. In the present study, administration of carbimazole (1.35 mg/kg) for a month resulted in a significant decline in serum levels of free T3, T4, testosterone and FSH levels while there was a significant increase in TSH & LH levels. These findings are concomitant with [43], who reported a significant reduction in serum levels of testosterone hormone induced by hypothyroidism with non-significant changes in LH and FSH serum levels. Similarly, another study showed that rats with hypothyroidism presented with high serum levels of LH hormone. Also, there was a significant decline in total serum testosterone levels. Their results highlighted the great role of thyroid hormones in the regulation of FSH biosynthesis [34]. Moreover, in hypothyroidism, the serum level of the LH hormone was increased, while serum level of testosterone was decreased [25]. The authors correlated these findings to the decrease in liver synthesis of SHBG in a hypothyroid state. SHBG is accountable for the transfer of testosterone from the testis to brain tissue, where free testosterone is dissociated and cross the blood-brain barrier (BBB), to induce negative feedback on LH biosynthesis and its secretion [26].

PTU-induced hypothyroidism significantly decreased serum levels of both T3 and T4 while, increased TSH level [36]. To better understand the pathological effects of hypothyroidism on testicular tissues, the oxidant-antioxidant state was studied in the testicular tissues. It seems that hypothyroidism can induce a disruption in the thiol redox status of mitochondria that resulted in testicular structural and functional abnormalities. Our data regarding these above-mentioned data showed a significant decrease in the GSH content in testicular tissue homogenate. Also, a decrease in sperm count and motility. However, a significant improvement in the level of GSH was recorded after ALA supplementation. This may aid in confirmation of the antioxidant system failure in the testes of carbimazole-treated rats. The results also revealed that carbimazole-induced hypothyroidism was able to increase MDA contents in the testicular tissue. In contrast, a partial improvement was noted in the carbimazole + α -lipoic acid group. The testes have a defense mechanism that is composed of the enzymatic and non-enzymatic antioxidant defense system, but its potential is limited. Furthermore, testes contain a high amount of polyunsaturated fatty acids (PUFAs) subjected to peroxidation by many oxidative radicals. In this regard, it has been proven that the production of high amounts of free radicals is harmful to sperm structure and function due to the bad effects on both the quality and quantity of DNA content of spermatozoa [7]. Taking into consideration these above-mentioned facts, the oxidative damage of testes tissue may play a main role in the obtained results of this study. The results obtained from previous reports indicated the alterations in thyroid hormone concentrations hurt mitochondrial GSH regulation mechanism, antioxidant defense mechanism, and their ratio to the functions of testes of adult rats [2]. On the other hand, the administration of ALA with carbimazole (1.35 mg/kg BW), resulted in a significant increase in GSH level, increase in sperm number and motility while a significant decrease in MDA testicular level was noted when compared with the carbimazole group. In agreement with our results, ALA is approved to be a potent factor to restore the deteriorated antioxidants in testicular rats exposed to cadmium [9].

CONCLUSIONS

Based on the obtained mentioned data, we can conclude that ALA as an antioxidant, can ameliorate the toxic effects of hypothyroidism on spermatogenesis and improved the structural and physiological processes in testes of hypothyroidism-induced rats. Furthermore, it can protect testicular tissues against oxidative damage. One of the limitations which we faced was the inability to add in situ hybridization as a confirmatory tool for our data.

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Table 1. Effects of carbimazole-induced hypothyroidism and α -lipoic acid on the histomorphometry and immunomorphometry of the testis in albino rats

Parameter	Control	α -lipoic acid	carbimazole	carbimazole + α -lipoic acid	ANOVA
Seminiferous tubule diameter (μ m)	241.80 \pm 34.47	242.90 \pm 29.80 ns	181.40 \pm 12.06 ***	218.7 \pm 5.554 *	< 0.0001 ***
Germinal epithelium thickness (μ m)	52.42 \pm 6.505	54.66 \pm 5.685 ns	29.26 \pm 3.760 ***	38.46 \pm 3.068 **	< 0.0001 ***
Interstitial space (μ m)	12.25 \pm 2.639	13.13 \pm 3.052 ns	6.74 \pm 1.346 ***	7.15 \pm 1.215 ns	< 0.0001 ***
Sertoli cell number / tubule	33.63 \pm 2.973	34.25 \pm 3.196 ns	11.13 \pm 2.232 ***	21.5 \pm 2.449 ***	< 0.0001 ***
Leydig cell number / cluster	14.75 \pm 1.282	15.13 \pm 1.885	2.38 \pm 1.408	9.88 \pm 1.458	< 0.0001

		ns	***	***	***
Androgen receptor area (%)	1.20 ± 0.345	1.13 ± 0.195	0.60 ± 0.119	0.93 ± 0.102	< 0.0001
		ns	***	*	***
Alpha smooth muscle actin area (%)	2.03 ± 0.388	2.09 ± 0.234	4.76 ± 0.305	3.88 ± 0.678	< 0.0001
		ns	***	**	***
Vimentin area (%)	14.63 ± 2.547	15.25 ± 1.949	7.74 ± 1.516	10.65 ± 0.611	< 0.0001
		ns	***	*	***

Values are expressed in Mean ± SD. 1-way ANOVA and post-hoc Bonferroni's multiple comparison test (α -lipoic acid and carbimazole vs control, carbimazole + α -lipoic acid vs carbimazole). *P<0.05, **P<0.01, ***P<0.001 and ns: no significance

Figure 1. A. Histogram showing thyroid function tests after different treatment strategies confirming the hypothyroid state. B and C: Histograms representing the effects of carbimazole-induced hypothyroidism and α -lipoic acid on gross measurements and semen analysis respectively. Data are presented as Mean ± SD. TSH: thyroid-stimulating hormone, T3: thyroxine 3 and T4 thyroxine 4. *P<0.05, **P<0.01, ***P<0.001 and ns: no significance.

Figure 2. Representative microphotograph of the testis in different groups. (A and B) Control group. The testis shows different types of cells LC: Leydig cells, ST: Sertoli cells, SG: Spermatogonia, SC: Spermatoocyte, and S: Spermatoid. (C and D) Hypothyroid group (carbimazole treated) the testis represents homogenous substance in the interstitial space (straight arrow), congestion (arrowhead), vacuolation (V), scarce Sertoli cells (ST), stripped off spermatoids (SS), scattered spermatoids (*) and detached germinal epithelium (D). (E and F) carbimazole + α -lipoic acid group expressing few Sertoli cells (ST), Leydig cells (LC), Spermatogonia (SG), Spermatoocyte (SC), Spermatoid (S) and detached germinal epithelium (D). (H&E, Bars = 200 & 50 μ m)

Figure 3. Representative microphotograph of the immunohistochemical expression in the testis. The groups are arranged as Control, carbimazole treated and carbimazole + α -lipoic acid-treated group respectively. (A, B and C: Androgen Receptor). The testis shows LC: Leydig cells, ST: Sertoli cells, and peritubular myoid cells (arrowhead). (D, E, and F: α Smooth Muscle Actin). The testis represents bilaminar peritubular myoid cells expression (arrowhead), thickening and fusion of the two laminae (arrow), and thickening in the interstitial vessel wall (*). (Bars = 50 μ m)

Figure 4. Representative microphotograph of vimentin expression in the testis in different groups. (A, B and C: Control, carbimazole treated and carbimazole + α -lipoic acid-treated group respectively). The testis shows different segments of Sertoli cells, a: apical segment and b: basal segment, destroyed detached apical segment of Sertoli cells (arrowhead), interstitial tissue (arrow) empty areas in the interstitial space (*). (Bars = 50 μ m)

Figure 5. Histograms representing the effects of carbimazole-induced hypothyroidism and α -lipoic acid on reproductive hormones, reduced glutathione, and MDA. Data are presented as Mean ± SD. FSH: follicle-stimulating hormone, LH: luteinizing hormone and MDA: malondialdehyde. *P<0.05, **P<0.01, ***P<0.001 and ns: no significance.









