Hypothyroidism impairs oxidant/ antioxidant status and testicular functions of adult male rats

Mervat E. Asker [a]; Wafaa A. Hassan [b] and Akram M. El-kashlan [b]

[a] Department of Biochemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.
[b] Department of Hormone Evaluation, National Organization for Drug Control and Research (NODCAR), Cairo, Egypt.
Dr.Mervatasker@hotmail.com, **Dr_wafaa_ahmed_hassan@yahoo.com**

Abstract: The present study describes the effect of hypothyroidism on testicular functions and oxidant / antioxidant status of adult male rats. Rats were made hypothyroidism by i.p. injection of 6-n propyl 2-thiouracil (PTU, 10mg/Kg body wt / day) for 4 and/ or 8 weeks. Induction of hypothyroidism caused a reduction in body weight as well as in genital sex organs weight. The epididymal sperm counts and their motility were significantly decreased in a time dependent manner following PTU treatment. A reduction in serum levels of Luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone, and an elevation in serum estradiol level were also noticed in hypothyroid rats. Significant increases in malondialdehyde (MDA), a lipid peroxidation marker, and nitric oxid (NO) levels were observed in testicular homogenate of hypothyroid rats. Increase in reduced glutathione (GSH) content and glutathione peroxidase (GPx) activity with a reduction in superoxide dismutase (SOD) and catalase (CAT) activities were also found following PTU-induced hypothyroidism. Marked histological changes were observed in the testicular section of hypothyroid rats. These results suggest a direct regulatory role of thyroid hormone on testicular physiology and oxidant / antioxidant state in adult animals.

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Key words: hypothyroidism; 6-n propyl 2-thiouracil; antioxidant; oxidative stress; sex hormones and testis.

Introduction:

Thyroid hormones, thyroxine (T4) and tri-iodothyroxine (T3), are involved in the regulation of numerous body functions consumption and several physiological functions such as development, reproduction and growth **(kundu *et al.,* 2006).** In mammals, altered thyroid status is known to adversely affect many organs and tissues. Nevertheless, for many years, the impact of thyroid disorders on male reproduction remained controversial. This was partly due to the demonstration that the adult testis of experimental animals was metabolically unresponsive to thyroid hormones **(Barker & Klitgaard, 1952),** and to the low thyroid hormone-binding sites found in the adult organ **(Oppenheimer *et al.,* 1974).** These early reports led to the widespread view that the testis was unaffected by iodothyronines. Additionally, clinical data correlating male sexual function with thyroid disorders are limited, probably because thyroid diseases are more common in females than in males.

The historical view of adult testis being unresponsive to thyroid hormone is negated by presence of thyroid hormone receptors and iodothyronine deiodinases throughout testicular development and in adulthood **(Wajner *et al.,* 2007).** Thyroid hormone deficiency during early stage of development has been shown to influence testicular physiology adversely, and impair antioxidant defense **(Sakai *et al.,* 2004; Sahoo *et al.,* 2008 and Zamoner *et al.,* 2008)**. However the effect of altered thyroid hormones upon testicular function vis-à-vis modulating oxidative stress in adult testis is not fully clear. Therefore, the aim of the current study is to find out whether thyroid hormone deficiency in adult life affects testicular functions by modulating the oxidant / antioxidant system.

Materials and methods

Chemicals

6-n propyl 2-thiouracil (PTU), thiobarbituric acid, reduced glutathione (GSH), Nicotinamide adenine dinucleotide phosphate (NADPH), glutathione reductase (GR), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), Folin's reagent, pyrogallol, sulfanilamide, N-(1-naphthyl) ethyl-enediamine, vanadium chloride (VCl_3) and bovine serum albumin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other used chemicals were of the highest analytical grades commercially available.

Experimental animals:

The experimental animals used in this study were adult male albino rats, weighing (250 ± 25g). The animals were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR), Egypt. The animals were housed in plastic cages allowed to adjust to the new environment for two weeks before starting the experiment. The rats were housed at 23±2°C and 55±5% humidity with 12 h light/dark cycle. Rats were fed on rodent chow and allow free access of drinking water. All animal procedures and the experimental protocols were approved by the Institutional Ethics Committee at NODCAR and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

Induction of hypothyroidism:

One week after acclimatization, 30 rats were rendered hypothyroidism by daily intraperitoneal (i.p.) administration of PTU, 10 mg/kg body wt **(Lee *et al.*, 2008 and Parmar & Kar, 2009)** for 4 weeks (the time period of two seminiferous epithelium cycles) and/or 8 weeks (the time period of one spermatogenesis). At the end of 4^{th} and 8^{th} week, blood samples were collected and processed for free T3 (fT3) free (fT4) and thyroid stimulating hormone (TSH) determination. The rats which achieved hypothyroidism as represented by significant increase in serum fT3 and fT4 and decrease in serum TSH compared to controlled groups, selected for this study as hypothyroid rats.

Experimental design

Rats were categorized as follow:

Control group: animals receiving a daily i.p. injection of alkaline saline for 8 consecutive weeks (n=30).

Hypothyroid group: animals receiving a daily i.p. injection of PTU (10mg/kg body wt) for 8 consecutive weeks (n=30). PTU was dissolved in alkaline saline.

Patches of 15 animals from each group were taken for blood collection and scarified after 4 and/or 8 weeks from daily i.p. treatment.

Body weight measurement:

Body weight was recorded daily beginning on zero time (the time prior to treatment) and continued until sacrifice. The body weight was averaged for each week until the end of the treatment.

Blood collection

After complete of treatment schedule, blood samples were collected from femoral vein and drown by vein puncture into serum separation tube after 4 and/ or 8 weeks. The blood was allowed to clot at room temperature for 30 minutes. Serum was then separated by centrifugation at 3000 revolution per minute (rpm) for 10 minutes at 4°C. The separated serum was collected and divided into aliquots, then stored at -20°C for further determination of thyroid hormones, sex hormones and total cholesterol.

Tissues preparation

The rats were sacrificed by decapitation after exposing them to mild ethyl ether anesthesia **(Parmer & Kar, 2009)**. pair of testes and epididymes, seminal vesicles (without the coagulating and full of secretion) and prostate gland were removed and cleared from adhering tissue, washed in ice- cold 1.15%KCL, pot dreied on filter paper and weighted. The right caudal epididimus was used for sperm count estimation, while the left one was used for sperm motility determination. The right testis was kept in 10% formalin for the histopathological examination, while the left testis was stored at -20°C for oxidative stress and antioxidant parameters determination.

Epididymal spermatozoal examination:

The epididymal content of each rat was obtained by cutting the tail of epididymus and squeezing it gently in sterile clean watch glass and examined according to the technique adopted by **Bearden & Fuquay (1997)** for the estimation of the following parameters.

a- Sperm cell count:

The spermatozoa were counted by hemocytometer using the Improved Neubauer (Deep 1/10mm. LABART, Germany) chamber. The undiluted semen was withdrawn up to the mark 0.5 of the erythrocyte pipette and then filled up to the mark 101 by normal saline stained with eosin. The contents of the pipette were mixed by holding the end of the pipette between the thumb and the index fingers and shaking it vigorously. The cover slide was placed over the counting chamber and a drop of diluted semen was spread between the haemocytometer slide and its cover. The sperms in 5 large squares (contain 80 small squares) were counted under a light microscope with 40 x objective lens. The sperm cell count determined as (10^6 / ml)**.**

b- Progressive motility:

A small droplet of semen was added to one drop of sodium citrate water solution (2.9 %) on a warm slide (sodium citrate solution and the slides kept at 37°C). Several fields were examined, and classified into motile and none motile sperms, and the incidences of progressively motile sperms were estimated and recorded as percentage (%) of sperm motility.

Hormone assays:

The level of free T3 (fT3), free T4 (fT4), thyroid stimulating hormone (TSH), Leuteinizing hormone (LH), Follicle stimulating hormone (FSH), testosterone and estradiol (E_2) were determined in serum samples by using ELISA kits (diagnostic systems laboratories INC.) supplied from (Monobind Inc., USA) according to the manufacturer's recommended protocol.

Total cholesterol level:

Total cholesterol was measured enzymatically as described by **Allain *et al.* (1974)**, using Elitech Kits (Elitech Diagnostics, France), following the instructions of manufactures.

Oxidative stress parameters

Lipid peroxidation was quantified as malondialdehyde (MDA) according to the method described by **(Simon *et al.*, 1994),** and expressed as nmol/g tissue. The nitric oxide (NO) content was quantified indirectly as nitrite concentration according to the method of **Miranda *et al.* (2001)** which depended on reduction of nitrate to nitrite by VCl_3, then the released nitrite was colorimetrically detected by Griess reagent. NO levels were expressed as µmol/g tissue.

Antioxidant parameters

The content of reduced glutathione GSH was evaluated by using the method of **Van Dooran *et al.* (1978)** which based upon the development of relatively stable yellow color when DTNB is added to sulfhydryl compounds. Superoxide dismutase (SOD) activity was determined by measuring the inhibition of auto- oxidation of pyrogallol at PH 8.5 according to **Nandi & Chatterjee (1988)**. The Catalase (CAT) activity was estimated from the ability of the tissue to decompose hydrogen peroxide (H_2O_2), whose concentration can be followed at 240 nm **(Aebi, 1984)**. Glutathione peroxidase (GPx) activity was assayed by measuring oxidation rate of NADPH in the presence of H_2O_2, GSH and GR **(Paglia & Valentine, 1967)**.

Determination of Protein

The protein content of the different tissue homogenates was determined according to the **Lowry *et al.* (1951)** method as modified by **Peterson (1977)** using bovine serum albumin as standard.

Histopathological examination

Testicular tissues for histopathological examination were fixed in 10% buffered formalin overnight and then embedded with paraffin. When analyzed, all paraffin-embedded tissue was sectioned at 4 µm, deparaffinized in xylene, dehydrated by ethyl alcohol in decreasing concentrations (100%, 95% and 70%), and stained with haematoxylin (Merck KGaA, Darmstadt, Germany) and eosin (Sigma, St. Louis, MO, USA). These specimens were examined under bright-field optical microscopy using a light microscope and × 40 magnification powers. Corresponding digital images were captured for later analysis **(Bancroft & Stevens, 1996).**

Statistical analysis

The quantitative data of continuous variables were expressed as mean ± S.D. Significance of mean values of different parameters between the groups were analyzed using Two Way Analysis of Variance (ANOVA) after ascertaining the leven test for homogeneity of variance between the treatments. Pair wise comparisons were done using (Tukey HSD and Gsmes-Howell) test. In case of non-homogeneity, a Welch and Brown Forsythe adjustment for the ANOVA. Person correlation coefficient was used to observe the relationship among the different variables. All analyses were performed using SPSS 18 for Windows (SPSS Inc., Chicago) and differences were considered statistically significant at probability level less than 0.05 for all tests.

Results:

1-Body weight

Figure (1) illustrates that, the changes in body weight of PTU treated animals were similar to that of control animals from zero time to two weeks of treatment, after that there were decreases in the body weight gain until the end of the time treatment schedule when compared with controls. Table (1) shows that rats received PTU demonstrated a significant (p<0.01) decrease in body weight gain after 4 and 8 weeks of treatment by (14.3% and 24.7%), respectively in comparison with control groups.

2-Thyroid hormone levels

Figure (2) illustrates that treatment of rats with PTU for 4 or 8 weeks significantly (p<0.01) decreased the levels of fT3 and fT4 and increased (p<0.05) the level of TSH in comparison with euthyroid rats. Relative to 4 weeks treatment groups, administration of PTU for 8 weeks produced a significant (p<0.01) reduction in fT3 by 21.1% while the other hormones (fT4 and TSH) recorded non-significant change.

3-Sex hormones

The data presented in Figure (3) show that administration of PTU for 4 or 8 weeks caused a notable decline in serum levels of LH, FSH and testosterone, however, yielded a significant (p<0.01) elevation in estradiol serum level relative to the control values. In comparison with 4 weeks hypothyroid animals, injection of PTU for 8 weeks displayed a significant (p<0.01) decrease in testosterone level (15.7%), and increase in estradiol level (57.9%).

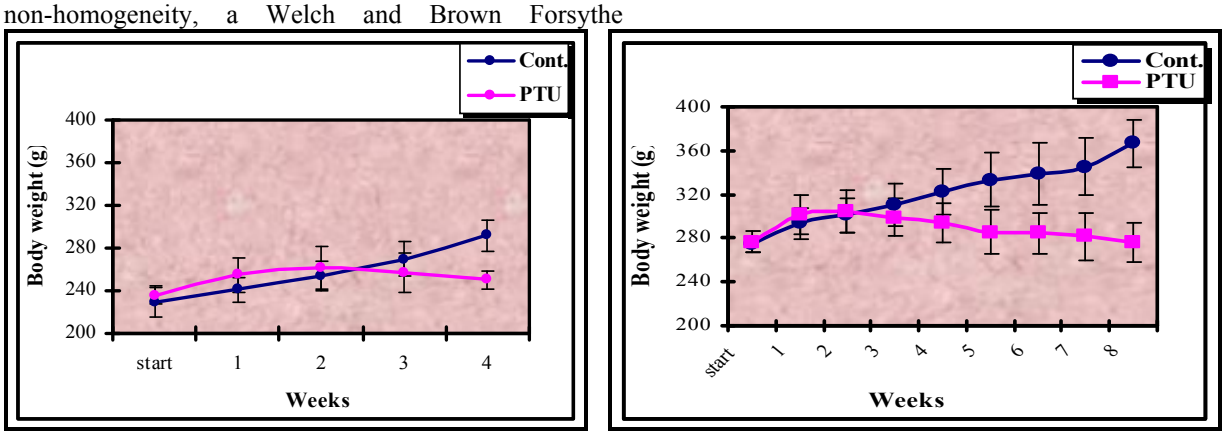

Figure (1): Time- related changes in body weight of adult male albino rats after 4 and/or 8 weeks of i.p administration of PTU (10mg/kg body wt/ day). Values are expressed as Mean ± S.D., (n=10).

Table (1): Effect of i.p administration of PTU (10mg/kg body wt/ day) for 4 and/or 8 weeks on body weight gain (g) of adult male albino rats. Values are expressed as Mean ± S.D., (n=10).

Time / Group	4 Weeks			8 Weeks		
	Initial	Final	%Difference	Initial	Final	%Difference
Control	229.40 ± 13.87	a+ 292.07 ± 14.61	27.3 %	273.90 ± 6.51	a+ 366.60 ± 22.13	33.8 %
PTU	235.50 ± 8.40	ab+250.25 ± 8.80	6.3 % (-14.3 %)	276.65± 9.67	b+ 276.05± 18.33	0.00 % (-24.7 %)

a: $p < 0.05$, a+: $p < 0.01$, significantly different from initial values.
b+: $p < 0.01$, significantly different from control values.
% difference: represents a comparison between initial and final body weight values.
( ) % difference: represents a comparison in final body weight between control and treated values.

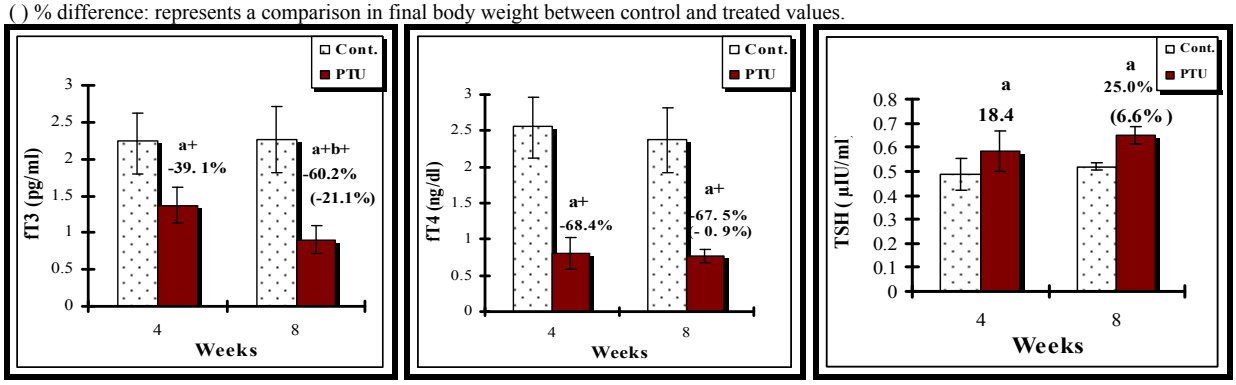

Figure (2): Effect of i.p administration of PTU (10mg/kg body wt/ day) for 4 and/or 8 weeks on serum levels of fT3 (pg/dl), fT4 (ng/ml) and TSH (µIU/ml) in adult male albino rats. Values are expressed as Mean ± S.D., (n=8).
a: $p < 0.05$, a+: $p < 0.01$ significantly different from control values.
b: $p < 0.05$, significantly different from 4-weeks treated values.
% difference: represents a comparison between control and treated values.
( ) : represents a comparison between % difference of 4 weeks-treated values and 8 weeks- treated values.

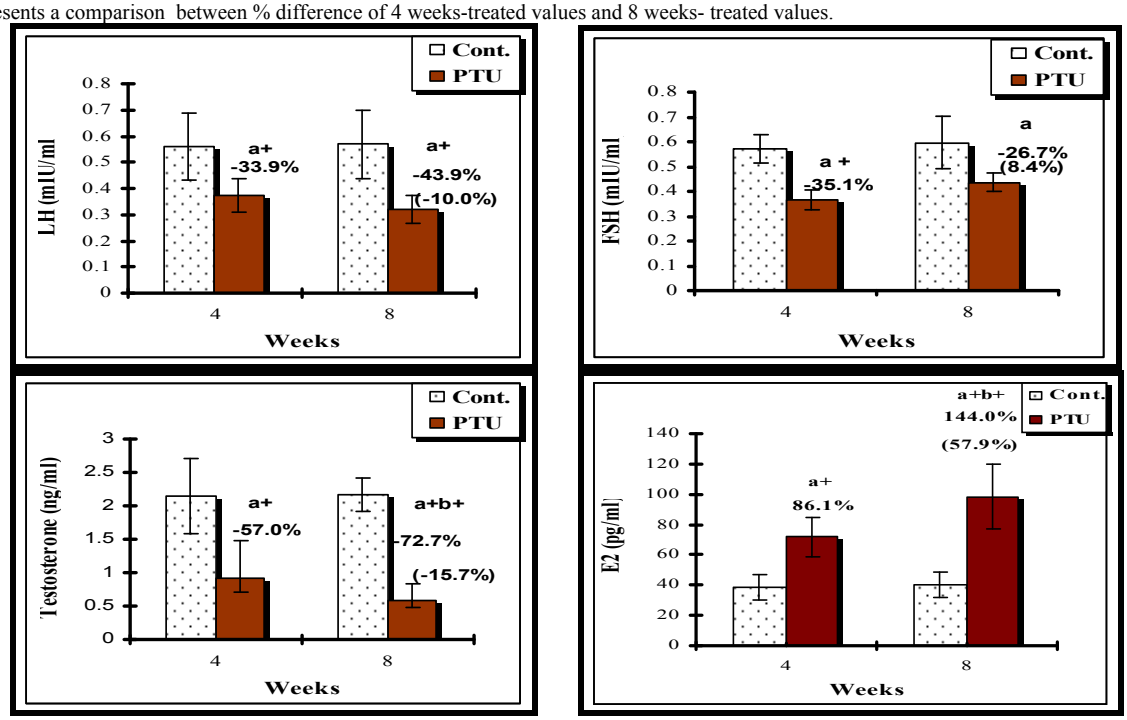

Figure (3): Effect of i.p administration of PTU (10mg/kg body wt/ day) for 4 and/or 8 weeks on serum levels of LH (mIU/ml), FSH (mIU/ml), testosterone (ng/ml), and E_2 (pg/ml) in adult male albino rats. Values are expressed as Mean ± S.D., (n=8).
a: $p < 0.05$, **a+:** $p < 0.01$ significantly different from control values,
b+: p< 0.01 significantly different from 4-weeks treated values.
% difference**:** represents a comparison between control and treated values.
( ) : represents a comparison between % difference of 4 weeks-treated values and 8 weeks- treated values.

4- Absolute and relative genital organs weight

The results summarized in Table (2) revealed the occurrence of a gradual, sustained highly significant decrease in the absolute weights of right and left testis, epididimus, prostate gland and seminal vesicle after 4 or 8 weeks of hypothyroidism induced by daily i.p. injection of PTU compared to control rats. Injection of PTU for 8 weeks caused 19.7%, 20.4% and 25.5% reduction in epididimus, prostate gland and seminal vesicle weights in comparison with 4 weeks hypothyroid rats, respectively. On the other hand, there are no significant changes in the relative weights of all genital organs observed in 4 weeks hypothyroid rats with the exception of a highly significant (p<0.01) decrease (29.4%) was noticed in prostate gland's relative weight in comparison with control animals. However, eight weeks hypothyroid rats exhibited a marked decline in relative weight of left testis, epididymus, prostate gland and seminal vesicle with respective to euthyroid rats. Propyl thiouracil administrated for 8 weeks induced a significant (p<0.05) reduction in relative weights of epididimus, prostate gland and seminal vesicle (16.7%, 20.6% and 22.1%), respectively relative to 4 weeks hypothyroid rats.

Table (2): Effect of PTU (10mg/kg body wt/ day) for 4 and/or 8 weeks on absolute and relative genital organ weights in adult male albino rats. Data are expressed as Mean ± S.D. of ten observations.

	4 weeks			8 weeks		
	Control	PTU	% difference	Control	PTU	% difference
Absolute organ weights (g)						
Right testis	1.47 ± 0.06	1.28 ± 0.11^{a+}	-12.9%	1.64 ± 0.13	1.34 ± 0.08^{a+}	-18.3% (- 5.4%)
Left testis	1.47 ± 0.06	1.26 ± 0.11^{a+}	-14.3%	1.63 ± 0.10	1.39 ± 0.09^{a+}	-14.7% (-0.4%)
Epididymus	0.18 ± 0.02	0.15 ± 0.02^{a+}	-16.7%	0.22 ± 0.02	0.14 ± 0.02^{a+b+}	-36.4% (-19.7%)
Prostate gland	0.49 ± 0.93	0.29 ± 0.05^{a+}	-40.8%	0.67 ± 0.17	0.26 ± 0.04^{a+b}	-61.2% (-20.4%)
Seminal vesicle	0.62 ± 0.10	0.46 ± 0.06^{a+}	-25.8%	0.80 ± 0.11	0.39 ± 0.09^{a+b+}	-51.3% (-25.5%)
Relative organ weights (g/100g B.W)						
Right testis	0.50 ± 0.04	0.51 ± 0.05	2.0%	0.45 ± 0.05	0.49 ± 0.04	8.9% (-6.9%)
Left testis	0.51 ± 0.04	0.50 ± 0.05	-2.0%	0.45 ± 0.03	0.51 ± 0.04^{a}	13.3% (15.3%)
Epididymus	0.06 ± 0.01	0.06 ± 0.01	0.0%	0.06 ± 0.01	0.05 ± 0.01^{a+b}	-16.7% (-16.7%)
Prostate gland	0.17 ± 0.03	0.12 ± 0.02^{a+}	-29.4%	0.18 ± 0.05	0.09 ± 0.02^{a+b}	-50.0% (-20.6%)
Seminal vesicle	0.21 ± 0.03	0.18 ± 0.02	-14.3%	0.22 ± 0.03	0.14 ± 0.04^{a+b}	-36.4% (-22.1%)

a: $p < 0.05$, a^{+}: $p< 0.01$ significantly different from control values.
b: $p < 0.05$, b^{+}: $p< 0.01$ significantly different from 4-weeks treated values.
% difference: represents a comparison between control and treated values.
( ) : represents a comparison between % difference of 4 weeks-treated values and 8 weeks- treated values.

5-Sperm parameters

Figure (4) clears that, rats received PTU for 4 or 8 weeks provoked a statistically significant (p<0.01), time dependent decrease in sperm count and sperm progressive motility relative to euthyroid rats. Eight weeks PTU treated groups exhibited a remarkable decrease in sperm count (15.2%) and motility (19.3%) in comparison with 4 weeks treated groups.

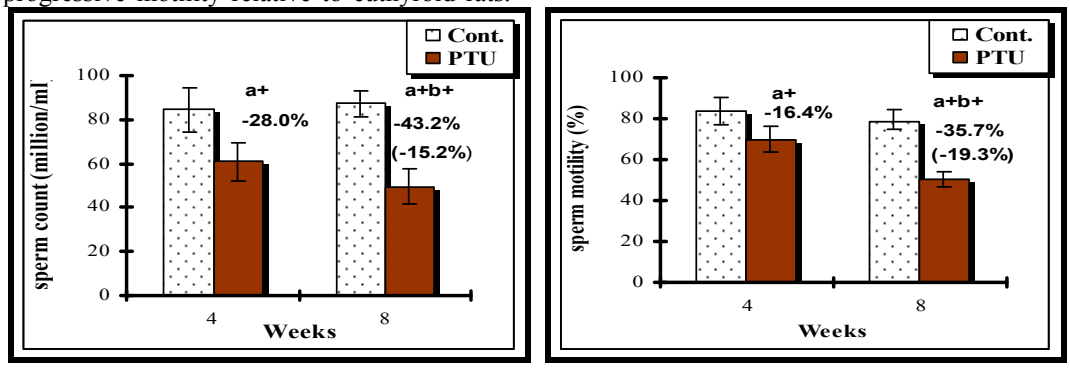

Figure (4): Effect of i.p administration of PTU (10mg/kg body wt/ day) for 4 and/or 8 weeks on epididymal sperm count (10^6/ml) and sperm motility (%) of adult male albino rats. Values are expressed as Mean ± S.D., (n=10).

a^{+}: $p< 0.01$ significantly different from control values.
b^{+}: $p< 0.01$ significantly different from 4-weeks treated values.
% difference**:** represents a comparison between control and treated values.
( ) : represents a comparison between % difference of 4 weeks-treated values and 8 weeks- treated values.

6-Oxidative stress parameters

As could be seen from Figure (5), MDA and NO levels were impressively (p<0.01) increased in a time dependent fashion after 4 or 8 weeks from hypothyroidism compared with euthyroid rats. Administration of PTU for 8 weeks showed a significant (p<0.01) elevation in MDA and NO concentrations (27.6% and 21.9%, respectively) compared to 4 weeks PTU treated groups.

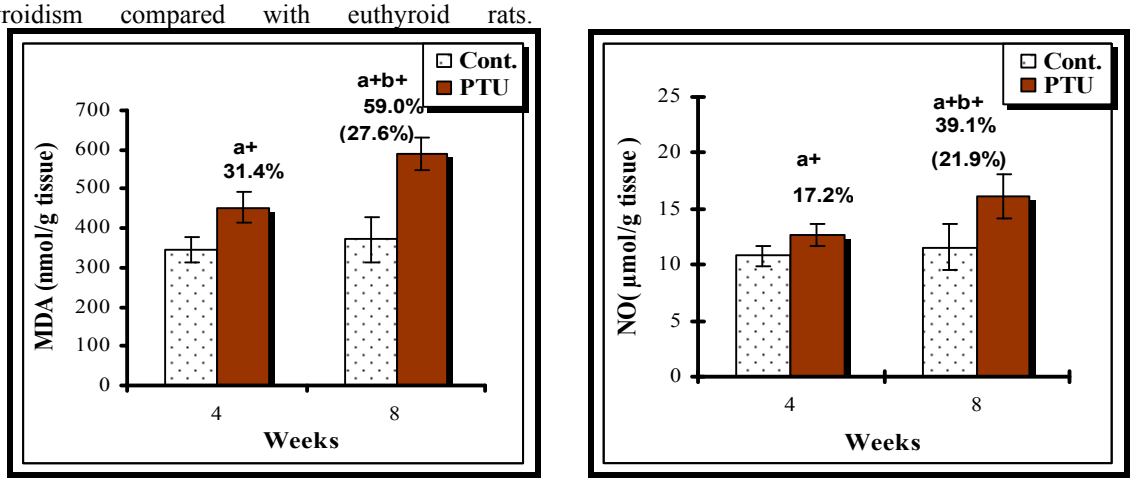

Figure (5): Effect of i.p administration of PTU (10mg/kg body wt/ day) for 4 and/or 8 weeks on testicular levels of MDA (nmol/g tissue) and NO (µmol/ g tissue) of adult male albino rats. Values are expressed as Mean ± S.D., (n=10).

a$^+$: p< 0.01 significantly different from control values.

b$^+$: p< 0.01 significantly different from 4-weeks treated values.

% difference: represents a comparison between control and treated values.

( ) : represents a comparison between % difference of 4 weeks-treated values and 8 weeks- treated values.

7-Antioxidant parameters

Figure (6) presents that rats received PTU for 4 or 8 weeks had a remarkable elevation in GSH content and GPx activity, while had a significant (p<0.01) decline in SOD and CAT activities relative to control rats. Induction of hypothyroidism for 8 weeks yielded a significant decrease in activities of GPx (29.7%, p<0.05) and CAT (36.1%, p<0.01) compared to 4 weeks hypothyroid rats.

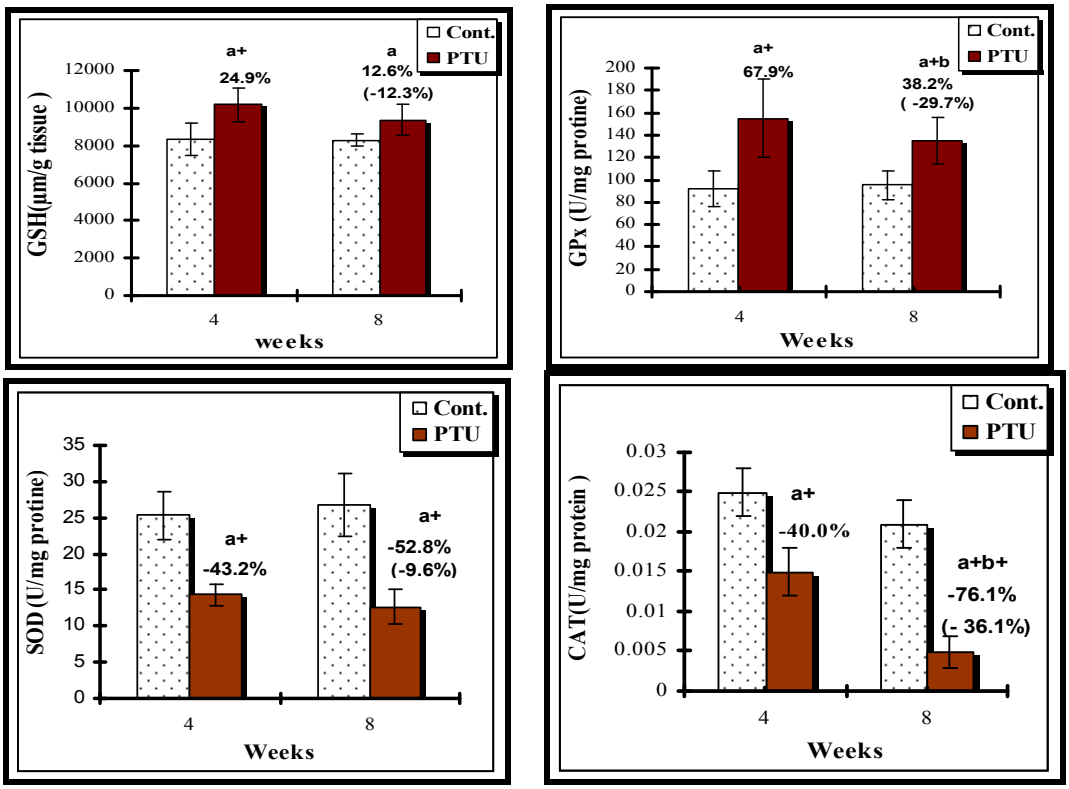

Figure (6): Effect of i.p administration of PTU (10mg/kg/ day) for 4 and/or 8 weeks on testicular GSH (µmol/g tissue), GPx (U/mg protein), SOD (U/mg protein) and CAT (U/mg protein) of adult male albino rats. Values are expressed as Mean ± S.D., (n=10).

a: $p < 0.05$, **a$^+$:** $p < 0.01$ significantly different from control values.
b: $p < 0.05$, **b$^+$:** $p < 0.01$ significantly different from 4-weeks treated values.
% difference: represents a comparison between control and treated values.
( ) : represents a comparison between % difference of 4 weeks-treated values and 8 weeks- treated values.

7-Cholesterol

Figure (7) demonstrate that cholesterol level significantly (p<0.01) increased by PTU administration for 4 or 8 weeks, with respective to euthyroid rats. Propyl thiourathyl administrated for 8 weeks caused notable (20.7%, p<0.05) increase in cholesterol concentration with compared to 4 weeks hypothyroid data.

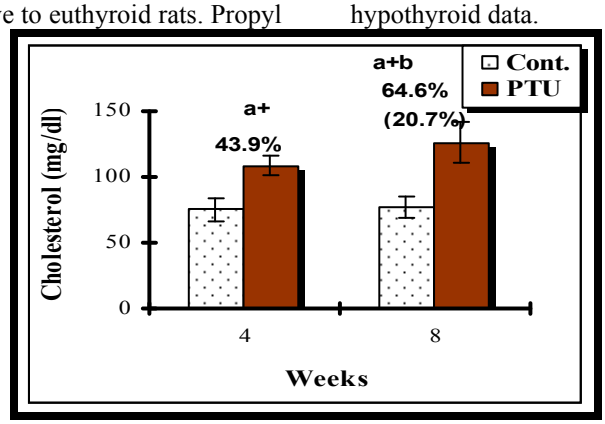

Figure (7): Effect of i.p administration of PTU (10mg/kg/ day) for 4 and/or 8 weeks on serum level of cholesterol (mg/dl) in adult male albino rats. Values are expressed as Mean ± S.D., (n=10).

a$^+$: $p < 0.01$ significantly different from control values.
b: $p < 0.05$ significantly different from 4-weeks treated values.
% difference: represents a comparison between control and treated values.
( ) : represents a comparison between % difference of 4 weeks-treated values and 8 weeks- treated values.

8- Correlation study

Using the combined results from all experimental animals, the present study demonstrate that epididymal sperm count and motility were significantly (p<0.01) positively correlated with the levels of testosterone (r = 0.684 and r = 0.558, respectively), LH (r = 0.732 and r = 0.618, respectively) and FSH (r = 0.684 and r = 0.558), but they were significantly (p<0.01) negatively correlated with the level of estradiol (r = -0.883 and r = - 0.850, respectively) (Table3).

Table (3): Correlation between sperm parameters (count and motility) and sex hormones.

	Testosterone	E_2	LH	FSH
Sperm count	0.684**	-0.833**	0.732**	0.684**
Sperm motility	0.558**	-0.85**	0.618**	0.558**

**** Correlation is significant at $P < 0.01$**
n = 32

Moreover, the data presented in Table (4) clear that epididymal sperm count, and motility showed a significant (p<0.01) positive correlation with SOD (r = 0.811 and r = 0.767, respectively) and CAT (r = 0.845 and r = 0.932, respectively) while they showed a remarkable (p<0.01) negative relationship with MDA (r = -0.828 and -0.830, respectively), NO (r = -0.737 and -0.831, respectively), GSH (r = -0.686 and r = -0.493, respectively) and GPx (r = -0.698 and r = -0.567, respectively).

The present study also demonstrate that testicular MDA and NO levels were significantly (p<0.01) positively correlated with testicular GSH (r = 0.464 and r = 0.421) and GPx (r = 0.559 and r = 0.532), while they were expressed a remarkable (p<0.01) negative relationship with SOD (r = -0.795 and r = -0.668) and CAT (r = -0.876 and r = -0.810, respectively), (Table 5).

Table (4): Correlation between sperm parameters (count and motility) and oxidative parameters (MDA and NO) and antioxidant parameters (SOD, CAT, GHPX and GSH).

	MDA	NO	SOD	CAT	GSH	GPX
Sperm count	-0.828**	-0.737**	0.811**	0.845**	-0.686**	-0.698**
Sperm motility	-0.830**	-0.831**	0.767**	0.932**	-0.493**	-0.567**

** Correlation is significant at P < 0.01
n = 40

Table (5): Correlation between oxidative parameters (MDA and NO) and antioxidant parameters (SOD, CAT, GHPX and GSH).

	SOD	CAT	GSH	GPx	NO
MDA	-0.795**	-0.876**	0.464**	0.559**	0.789**
NO	-0.668**	-0.810**	0.421**	0.532**	1

** Correlation is significant at P < 0.01
n = 40

Histological studies:

Histological examination of testicular section of control animals showed multiple rounded seminiferous tubules with regular outlines. They were lined by 4-6 layers of germinal epithelium at different stages of spermatogenesis. The flagella of mature sperms, which had whirly appearance, were seen filling the lumina of the seminiferous tubules. The interstitial spaces in-between the tubules contained Leydig cells and some blood capillaries (plate 1).

Microscopically examination of testicular tissues of 4 weeks PTU treated group's revealed pathological alterations in form of spermatogenic arrest in the seminiferous tubules (plate 2). After 8 weeks more damage in testis tissues is seen, where there is spermatogenesis arrest in the lumen of the most tubules and thickening and oedema in interstitial spaces. The lumen of few seminiferous tubules occupy with eosinophilic a cellular mass occasionally seen (plates 2).

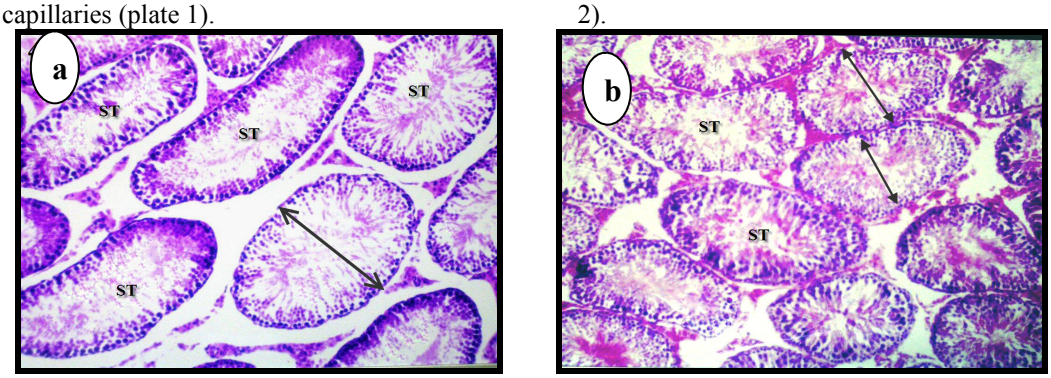

Plate (1): A photomicrograph of testis in control groups for 4 (a) and 8 (b) weeks showing normal histological structure of mature active seminiferous tubules with complete spermatogenic series (ST). (H&E x 40).

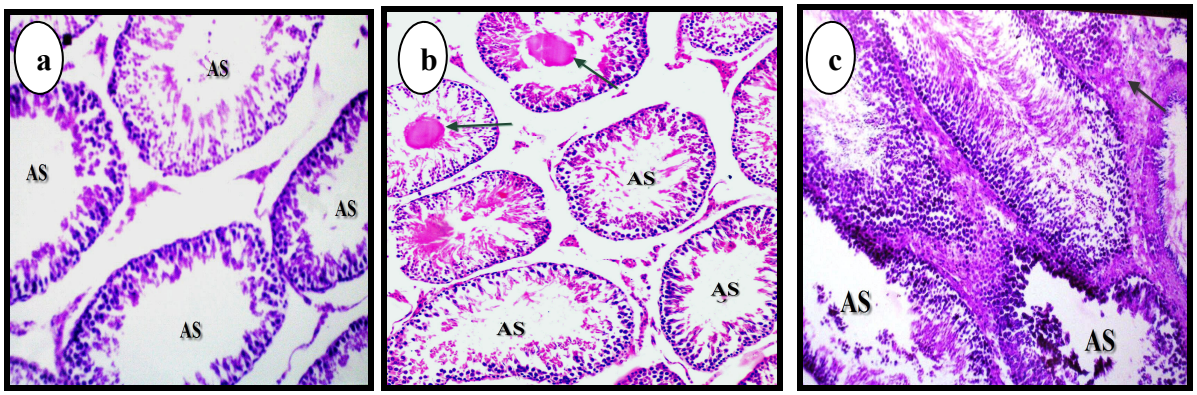

Plate (2): A photomicrograph of rat testis of (a) treated with PTU for 4 weeks showing spermatogenic arrest in the lumen of the most tubules (AS), (b) treated with PTU for 8 weeks showing spermatogenic arrest in the lumen of the most tubules (AS) and seminiferous tubules lumen occupied with eosinophilic acellular mass, and (c) treated with PTU for 8 weeks showing thickening and oedema in interstitial spaces (arrow). (H&E x 40).

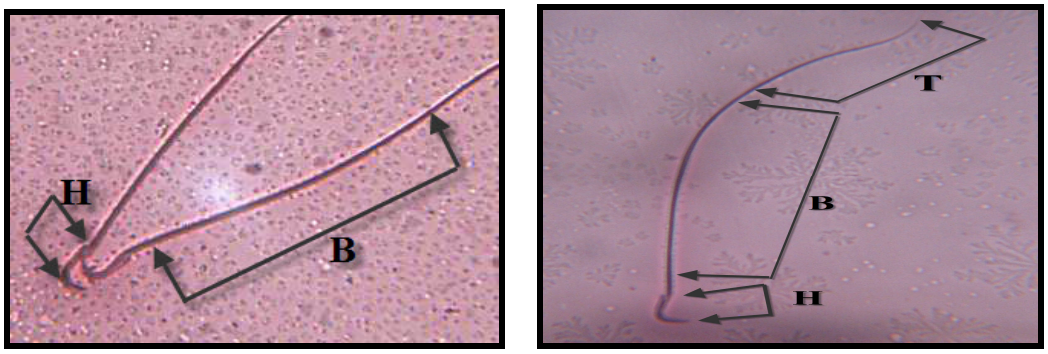

plate (3): Showing the normal sperm with head (H), body (B) and tail (T).

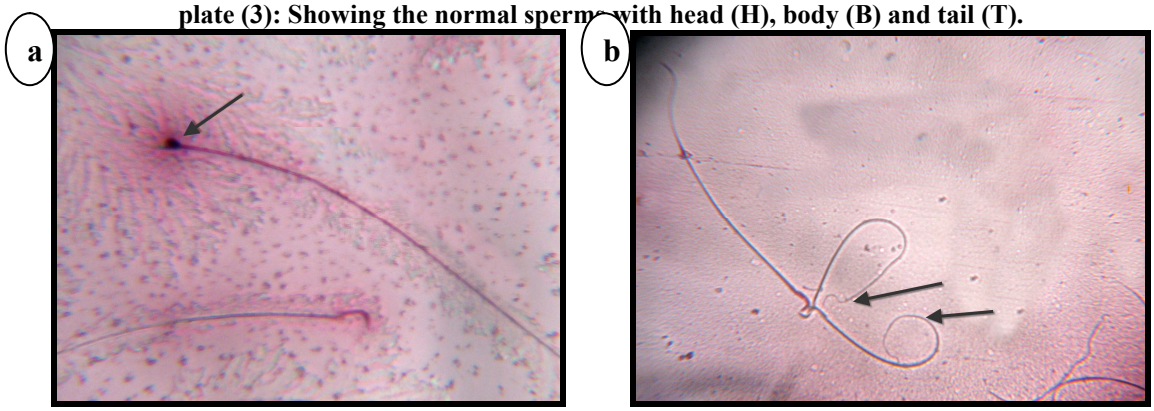

Plate (4): Effect of PTU administration on different forms of sperm abnormities in male rats, showing detached head sperm (arrow in a) and coiled tail sperm (arrow in b)

Discussion

From the present study it could be noticed that induction of hypothyroidism by i.p. injection of PTU was confirmed by a decrease in body weight gain after 4 and 8 weeks (Table1). Our results are in accordance with earlier report that showed an association of decreased body weight with hypothyroidism of adult rats **(Jiang *et al*., 2000; Choudhury *et al*., 2003 and Sakr *et al*., 2011). Sahoo *et al*. (2008)** recorded that thyroid hormone is essential for the growth of animals and a significant reduction in the body weight of PTU treated rats in comparison to their respective control suggest that disruption of normal thyroid hormone levels in animals by PTU is the reason for the same.

In the present study the effectiveness of PTU administration in inducing hypothyroidism was also verified by a pronounced decrease in serum concentration of fT3 and fT4 associated with an increase in TSH circulating level (Table 1) as compared to euthyroid rats. These findings are similar to those obtained by several investigators **(Sahoo *et al.*, 2007; Parmar & Kar, 2009 and Subudhi *et al*., 2009).**

Zoeller & Crofton, (2005) suggested that PTU administration inhibit thyroperoxidase activity within the thyroid gland leading to block the organification of thyroglobulin and thus, block thyroid hormones

synthesis. Treatment with PTU inhibited the oxidation of iodide to oxidized iodine and its binding to tyrosine moieties in thyroglobulin: a central step ithyroid hormone synthesis catalyzed by the thyroperoxidase and decreasing Di deiodinase's activity and peripheral transformation of T4 **(Taurog, 1986).** Moreover, **Scott-Moncrieff *et al.* (1998)** indicated that the increasing effect of PTU on TSH level may be due to the lack of negative feed back from the thyroid hormone.

In the present study, serum levels of LH, FSH and testosterone were reduced in hypothyroid rats (Fig.1). The above observation was run parallel with finding of **Hardy *et al.* (1993)** who reported that hypothyroidism results in significant decrease in LH and FSH and also a fall in serum testosterone concentration**.** Moreover, **Choudhury *et al.* (2003) and Sakr *et al.* (2011)** reported decline in LH serum level of hypothyroid rats. The decrease in LH may be due to reduction in the release of gonadotropine-releasing hormone (GnRH) as a result of hypothyroidism **(Kirby *et al.*, 1997; Tohei *et al.,* 1997 and Chiao *et al.,* 1999)**. Also The LH and FSH gonadotropins are synthesized and secreted by the gonadotropic cells in response to the GnRH **(Rago & Salacone,2008),** so depending on the above suggestion, the decrease in FSH level may be due to decline in the release of GnRH as a result of hypothyroidism.

The decrease in the serum level of testosterone was recorded following hypothyroidism in the present study. Previous studies have shown that PTU **(Choudhury *et al.*, 2003),** carbimazole, antithyroid agent**, (Sakr *et al.,* 2011)** treatment and thyroidectomy **(Oncu *et al.*, 2004)** decrease serum testosterone secretion in the rats. Moreover, **Donnelly & White (2000)** noticed a decline plasma testosterone level in hypothyroid rat, which was rose after commencement of T4 therapy. Reduced serum gonaotropins and testosterone levels were also observed in congenital hypothyroid *rdw* rats (**Sakai *et al.,* 2004**). Such decrease in testosterone level may be due to a decrease in serum LH level. It is a well established fact that LH stimulated testosterone synthesis in Leydig cells. The above suggestions agree well with the results of the present study where the decrease in LH level was accompanied by a decrease in testosterone serum level in hypothyroid animals. Moreover, **Ai *et al.* (2007**) recorded that lowered serum testosterone concentration in hypothyroid rats was attributed to the decrease number of Leydig cells and also could be due to the effect of thyroid hormones on steroidogenic function of Leydig cells. Leydig cells from PTU-treated rats produce less testosterone than controls following incubation with hydroxycholesterol, a testosterone precursor **(Hardy *et al.*, 1993)**.

Other mechanisms, in addition to the deficiency of LH release are suggested to be involved in regulating testosterone secretion in hypothyroid status. **Chiao *et al.* (2000) & (2002)** concluded that PTU decreased activity of cytochrom P450 side chain cleavage (P450scc) enzyme, the rate limiting enzyme in gonadal steroidogenesis, and which regulated by the gonadotropins and also diminished the mRNA expression of StAR (a protein which has been identified as being involved in the acute regulation of steroid production in steroidogenic tissues), and that this modulates the steroidogenesis of testosterone. These thyroid hormone-independent results seem to indicate that PTU act directly on steroidogenic organs.

The present data also demonstrated that hypothyroidism induced by PTU produced a dramatic increase in serum estradiol levels. **Panno *et al.* (1994)** reported that hypothyroidism is associated with an increase in estradiol secretion**.** The author also added that the increase in estradiol serum level following hypothyroidism may reflect increased synthesis of this hormone. This suggestion was confirmed by previous studies which reported an increase in aromatase activity, the key enzyme responsible for estrogen biosynthesis, **(Carreau *et al.*, 2004)**, in hypothyroid rats**. Rasheeda *et al.* (2005)** also showed an increase in the expression of aromatase transcript, which may leads to an increase in estrogen content in hypothyroid state **(Kuiper *et al.*, 1997).**

The present work revealed that, treated rats with PTU daily for 4 and/or 8 weeks caused reduction in the weight of genital sex organs (right and left testis, epididiymus, prostate gland and seminal vesicle) and induced many histological alteration including disorder in spermatogenesis in the lumen of most tubules, thickening and oedema in interstitial space, and presence of eosinophilic cellular mass in the lumen of some seminiferous tubules. The weight of the testis is largely depending on the mass of the differentiated spermatogenic cells; the reduction in the weight of testis may be due to decrease number of germ cells, decline in spermatogenesis and steroidogenesis **(Chapin *et al.,* 1997 and Takahashi & Oishi, 2001).** The observed weight loss of accessories sex organs may be due to reduced bioavailability of sex hormones such as testosterone, LH and FSH, the level of which in circulation indicate the reproductive endocrine status of the male **(Schrad, 2003).**

The results of the present study also declare that induction of hypothyroidism significantly decreased sperm counts and their motility which agree well with the results of previous studies **(Ai *et al.,* 2007 and Sahoo *et al.,* 2008).** It will not be out of context to mention that on a majority of species both testosterone and FSH are required to ensure normal spermatogenesis **(Weinbauer & Wessels, 1999)**. In accordance with the above suggestion, a significant decrease in the count of sperms in hypothyroid rats observed in the present study

indicate the damaging effect of PTU on normal spermatogenesis, which is further confirmed by the accompanying low serum testosterone and FSH levels. It has also been reported that hypothyroid animals exhibit altered protein binding of testosterone in plasma leading to decrease number and forward motility of sperm recovered from the cuda epididymus **(Del Rio *et al.*, 2001).**

The function of the epididymal epithelium, including absorption, secretion, synthesis, and metabolism, produce an appropriate luminal environment for the acquisition of fertilizing ability and motility of spermatozoa **(Hinton, 1990 and Turner, 1991).** Results of several studies have shown that hypothyroidism induces epididymal hypofunction is associated with decreased sperm motility **(Chandrasekhar *et al*., 1986; Kumar *et al*., 1994 and Del Rio *et al*., 1998).** The above suggestion agrees with the present study where induction of hypothyroidism leads to reduced epididymal weight and sperm counts and their motility.

Spermatozoa have been considered to be highly susceptible to lipid peroxidation in the presence of elevated reactive oxygen species (ROS) level, due to abundance of poly unsaturated fatty acid (PUSFA) in their membranes **(Peltola *et al.*, 1992).** Increase ROS formation due to lipid peroxidation and compromised antioxidant defense system may be caused mid-piece abnormalities and decreased sperm count and motility **(El-Taieb *et al.*, 2009 and Shiva *et al.*, 2011)**. This suggestion is confirmed by the present results which show a significant negative relationship between MDA with sperm counts and their motility as well as significant positive relationship between SOD and CAT with sperm counts and their motility (Table 4).

The induction of oxidative stress in hypothyroid rats in the present study is reflected by increased production of TBA-RS, a measure of lipid peroxidation in the homogenates of testis. The enhancement of lipid peroxidation might be due to oxidation of membrane lipids of different spermatogenic cells as it is reported that thyroid hormone affects almost all pathways of lipid metabolism **(Hoch, 1988).**

Mitochondrial respiration is the primary physiological source of ROS and is modulated by thyroid hormones. Hypothyroidism is associated with decrease in mitochondrial oxygen consumption and ATP synthesis **(Venditti *et al.*, 2003)**, especially in state III and IV of respiratory chain **(Subudhi *et al*., 2009 and Chattopadhyay *et al*., 2010).** Mitochondrial production of ROS mostly occur during sate IV respiration **(Chance *et al*., 1979)** particularly in the proximity of complex I and complex III of electron transfer chain **(Andreyev *et al*., 2005)**. Inhibition of complex I and III in hypothyroid rats **(Franco *et al.*, 2006)** might increase electron leakage leading to generation of more ROS especially H_2O_2. **Choudhury *et al.* (2003)** and **Chattopadhyay *et al.* (2010)** found that hypothyroidism induced by PTU in adult male rats lead to increase H_2O_2 level in testis. The reaction of $O^{\cdot}{}_2$ and H_2O_2 or free iron resulted in formation of $\cdot OH$ or ferryl iron which initiate lipid oxidation by hydrogen abstractyion of PUSFA **(Aust *et al.*, 1985**), resulting in membrane damage.

Testis being rich in PUFAs **(Peltola *et al.*, 1992)** is more prone to peroxidative agents. Also hypothyroidism can modify the acyl composition in plasma membrane by increasing the synthesis of PUFAs **(Hoch, 1988).** High PUFAs content together with presence of heme proteins and non-heme iron (catalysts for lipid peroxidation) in the testicular mitochondrial membrane pose continuous challenge by oxidant stress **(Rosenblum *et al.*, 1985).** This was reflecting in augmented TBARS level in the testicular homogenate. This is supported by evidences of increased lipid peroxidation in adult testis **(Sahoo *et al*., 2008; Chattopadhyay *et al*., 2010 and Sakr *et al*., 2011)** of hypothyroid rats.

The present results also show that hypothyroidism induced by PTU connected with the increase in the level of testicular NO. The data of several investigators illustrated that hypothyroidism induced the expression and activation of mitochondrial nitric oxide synthase (mtNOS), that complete reversed by hormonal replacement. Thus high mtNOS content conduct to excess synthesis of NO **(Carreras *et al.*, 2004 and Franco *et al.*, 2006).** It was reported that NO tonically modulate O_2 uptake by reversible inhibition of cytochrome oxidase and complex III through heim iron nitrosylation leading to inhibition of electrons transfer to O_2 to form H_2O and respiration in rats and promote formation of superoxide anion and hydrogen peroxide **(Poderoso *et al*., 1996 & 1999 and Brookes *et al*., 2003)**.

At higher NO concentration, NO and derived $O^{\cdot -}{}_2$ react to form peroxinitrite ($ONOO^-$) which is a powerful oxidizing and nitrating agent **(Carreras *et al.*, 2004).** Harmful $ONOO^-$ oxidant inhibit NADH dehydrogenase activity at mitochondrial complex I by nitrosylation or nitration leading to reduce O_2 up take **(Poderoso *et al.*, 1999 and Franco *et al*., 2006).** The results which are consistent with the data of the present study, which show a statistically significant, time dependent, positive correlation between MDA and NO (Table 5).

In the present study, a high lipid peroxidation with a concomitant decrease in the enzymatic antioxidant status, SOD and CAT were recorded in testicular tissue of hypothyroid rats. These enzymes are closely related to direct elimination of ROS **(Sahoo *et al.*, 2008)**. Therefore, the reduction in the activity of these enzymes may be contributed to increase utilization of these enzymes in scavenging and neutralizing the free radicals and lipid peroxides that are generated due to hypothyroidism **(Hulbert,**

2000; Das & Chainy, 2001&2004; Sarandol *et al.,* 2005 and VijayaKumar & Nalini 2006)

Several investigators observed an elevation in testicular level of H_2O_2 in hypothyroid rats **(Choudhury *et al.,* 2003 and Chattopadhyay *et al.,* 2010)**. Therefore, elevation of GPx activity in hypothyroid rats observed in the present study may be due to increase the biosynthesis of enzymes to compensate the H_2O_2 and radical over production, where GPx is the primary responsible for removal of testicular H_2O_2. Thus, the increase in GPx activity with the high level of MDA may be an indicator of the failure of compensating the induced oxidative stress. These notions were supported by the findings of the present study where a statistically significant, time dependent positive correlation was observed between GPx with MDA and NO (Table 5).

In the present study the effect of hypothyroidism on reduced glutathione was investigated. Mammalian testis contain relatively high level of GSH **(Calvin & Turner, 1982)**, which is reported to play an important role in the proliferation and differentiation of spermatogenic cells **(Knapen *et al.,* 1999)** by protecting them from noxious effect of ROS and maintain thiol level of protein **(Teaf *et al.,* 1985 and Sies, 1999),** where protein oxidation can lead to loss of critical thiol groups **(Pready *et al.,* 1998).** The maintains of free protein sulphhydryl group is important in proper folding and activity of protein. The elevated GSH pool in response to hypothyroidism may be an adaptation mechanism by organ to defence against over production of free radicals and oxidative stress (**Sahoo *et al.,* 2008).** When GSH present in extracellular space, it able to react directly with cytotoxic aldhyde produced during lipid peroxidation and thus protect the free sulphydryl group on the sperm plasma membrane **(Eskiocak *et al.,* 2005).** GSH is a cofactor in the GPx mediated destruction of hydroperoxidase, which protect the cell membrane against oxidative damage by regulating the redox state of protein in cell membrane **(Varghese & Oommen, 1999 and Varghese *et al.,* 2001)**. Therefore, the increased GPx in hypothyroid state might be one of the key factors responsible for elevate GSH level.

In conclusion, this study indicates that thyroid hormone plays an important role in maintaining of normal testicular function of adult animals. This report also provide evidence that alteration in thyroid hormone level is known to adversely affect the physiological function of adult testis by modulating its oxidant / antioxidant status as well as via alteration in sex hormone levels.

The data obtained from the present study show that hypothyroidism caused a significant increase in total cholesterol serum level. and this was compatible with several investigators who recorded an increase in total cholesterol serum level in adult hypothyroid rats **(Messarah *et al.,* 2010)** and serum of hypothyroid patients **(Nanda *et al.,* 2008a)**.

Hypercholesterolemia might have resulted from increased mobilization of body fat reserves as a result of increased thyrotropic hormone level-induced hypothyroidism. Low thyroxin level in hypothyroid also might have stimulated corticotrophin, and in turn, adrenal steroids, thereby might have increased lipid mobilization through over lapping endocrine axis **(Messarah *et al.,* 2010).** Moreover, hypercholesteremia and poor body weight have been suggested as excellent indicators of suspected hypothyroidism **(Al-Tonsi *et al.,* 2004).**

Thyroid hormones upregulate LDL receptor expression. Thus, the low concentrations of T3 and T4, found in hypothyroidism promote a reduction in the catabolism of lipoproteins leading to hypercholesterolemia **(Mayer *et al.,* 2008)**. High serum lipids are associated with lipid peroxidation as well as with oxidative stress damage in hypothyroidism because hypercholesterolemia provides a pool of substrates to be oxidized by free radicals **(Nanda *et al.,* 2008b)**. In this study, TBARS were significantly higher in rats with hypothyroidism, showing direct damage to lipid structures via free radicals in hypothyroidism. Previous studies have also demonstrated an increase in lipid peroxidation markers in overt hypothyroidism **(Torun *et al.,* 2009).**

In conclusion, this study indicates that thyroid hormone plays an important role in maintaining of normal testicular function of adult animals. This report also provide evidence that alteration in thyroid hormone level is known to adversely affect the physiological function of adult testis by modulating its oxidant / antioxidant status as well as via alteration in sex hormone levels.

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