

Effect of an anti-thyroid drug, 2,8-Dimercapto-6-hydroxy purine on reproduction in male rats

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Abstract: This histomorphological study is designed to evaluate the peripheral action of 2,8-Dimercapto-6-hydroxypurine (an antithyroid drug) on male reproductive system. The drug was administered as i.p. injection for 21 days to investigate its role on morphology of intratesticular cells and plasma testosterone level. Adult male rats (n=12), divided into three groups i.e. control, dimethylsulphoxide (DMSO) and 2,8-Dimercapto-6-hydroxypurine treated groups and treated with saline, DMSO and 2,8-Dimercapto-6-hydroxypurine for 21 consecutive days respectively. Blood samples were collected at day 1, 7, 14 and 21 and analyzed by using EIA systems. All the animals were scarified on 22nd day and testicular tissues were studied by histomorphological assesment. 2,8-Dimercapto-6-hydroxypurine caused a significant decrease (P<0.0001) in mean testicular cell population, testicular cell diameter and resulted in arrested spermatogenesis. A significant decrease (P<0.0001) was observed in mean Sertoli and Leydig cell population and diameter in treated group. Similarly a significant decrease was observed in plasma testosterone levels at days 1, 7 and 14 (P<0.05) and further decrease by day 21 (P<0.01) of drug treatment. The present study suggests that 2,8-Dimercapto-6-hydroxypurine is a negative modulator of reproductive system as it suppressed the plasma testosterone level and proliferation of different testicular cell types in adult male rats.

Keywords: Antithyroid drug, testes, testosterone, rats.

INTRODUCTION

Antithyroid drugs having simple structure are known as thionamides. These molecules contain a sulfhydryl group and a portion contains thiourea with a heterocyclic structure. Propylthiouracil (PTU) (6-propyl-2-thiouracil) and methimazole (MMZ) (1-methyl-2-mercaptoimidazole, Tapazole) are frequently used as antithyroid drugs and thyroid gland concentrate them against concentration gradient (Marchant *et al.*, 1971). Their major function is to reduce thyroid hormone synthesis by its inhibitory action on thyroid peroxidase-mediated iodination of tyrosine residues in thyroglobulin. This is very important step in thyroxine and triiodothyronine (Cooper, 2005).

Thyroid hormone level can severely affect reproductive functions including fertility, pregnancy and postnatal development in humans and rat (Chaio *et al.*, 2000). It was observed that these hormones play a vital role in male but not in female reproductive system in humans and rodents (Jannini *et al.*, 1995). Neonatal hypothyroidism studies in humans and experimental animals explained that it is associated with delayed puberty and abnormal gonadal function. It may be concluded that these hormones are involved in gonadal development and function (Cristovao *et al.*, 2002). Arrested spermatogenesis, decrease in numbers of Sertoli cells, Leydig cells and other testicular abnormalities in

hypothyroidism. These abnormalities were corrected by the injection of thyroxine (T4) (Tahmaz *et al.*, 2000). Thyroid hormone control many functions of Sertoli cells (Manna *et al.*, 1999).

It was reported that PTU treatment caused 80% and 140% increase in testis weight and daily sperm production respectively (Jansen *et al.*, 2007). In these animals these changes were due to increased cell populations including Sertoli, Leydig and germ cells. In these animals steroidogenesis by Leydig cells and gonadotrophin production by the pituitary were reduced permanently. After the first 10 days of birth PTU treatment in rats proved to be effective in disturbing the function of neuroendocrine-gonadal axis in adults. Both primary hypothyroidism and hyperthyroidism have been well documented as producing variable degrees of gonadal dysfunction (Dermott, 2004). When exposed to PTU, delayed spermatogenesis was observed with increase in apoptosis of spermatocytes. It was also concluded that after maternal exposure to antithyroid drugs from the mid-gestation to the end of lactation caused delay in onset of puberty and enlargement of gonads in adult stage. This result was considered due to systemic growth retardation lasting into the adults especially in males (Shibutani *et al.*, 2009).

The present study was designed to investigate the effect of a newly synthesized antithyroid drug 2,8-Dimercapto-6-hydroxypurine on testosterone secretion and testicular cells proliferation in adult male rats.

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MATERIAL AND METHODS

Animals

Adult male Sprague-Dawley rats of 60 days old, weighing 200-250 gm were used and kept at room temperature in animal house at Quaid-i-Azam University, Islamabad. The rats were fed with pelleted food and water was available ad libitum. The rats were housed in groups in steel cages containing four rats in each group per cage. The individual rat was identified by a tail tag. The rats were monitored and maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Experimental design

The animals were divided into three groups each group containing four rats i.e., control, vehicle and antithyroid drug 2,8-Dimercapto-6-hydroxypurine treated rats. The control group was administered with saline, vehicle group was administered with DMSO and treated group received 2, 8-Dimercapto-6-hydroxypurine dose intraperitoneally i.e. 20 mg/kg for 21 days daily in the morning. A stock solution of the antithyroid drug was obtained by dissolving powdered 2,8-Dimercapto-6-hydroxypurine in DMSO and stored at room temperature (25°C). The animals were scarified on 22nd day of treatment under deep diethyl ether anesthesia. The testes were removed for histomorphological studies.

Blood sampling

The blood samples were obtained on day 1, 7, 14 and 21 from each animal under light diethyl ether anesthesia. Blood (0.5 ml) was withdrawn from tail vein through insertion of heparinized syringe from each rat. The blood samples were centrifuged at 3000 rpm for 10 minutes. Plasma was separated and stored at -20°C until its analysis.

Tissue preparation and histology

Testes were surgically removed by incising the scrotal sac. Collected whole testes were weighed. Tissues were then immersed in fixative sera. Following dehydration in the descending ascending grades of ethyl alcohol, tissues were clarified in cedarwood oil and then embedded in paraffin. The 5µm thick sections were cut out of paraffin block by using Reichert Microtome. Sections were then affixed to pre-cleaned albuminized glass slides and stretched at 60°C on Fisher slide warmer. Hematoxylin and Eosin (H&E) staining was carried out and the slides were then examined under a Nikon optishot research microscope equipped with an automatic micro photographic system (Leica, Germany). 12 to 15 seminiferous tubules were studied in each slide and the mean number and nuclear diameter of different cell types in one tubule in the control, DMSO and treated groups were recorded.

Hormonal analysis

Plasma testosterone was quantitatively determined using Enzyme Immuno Assay kits (Amgenix International Inc., USA) according to the manufacturer instructions.

STATISTICAL ANALYSIS

Data were expressed as mean±SEM. One-way ANOVA (followed by Tukey's test) was applied to analyze the data. Statistical significance was set at $P \leq 0.05$.

RESULTS

Plasma testosterone concentrations

Anti-thyroid drug treatment caused a significant ($P < 0.05$) decrease after day 1, 7, 14 and highly significant decrease ($P < 0.01$) after day 21 in plasma testosterone concentrations as compared to control group. DMSO treatment also caused a significant decrease ($P < 0.05$) in plasma testosterone concentrations after day 14 and day 21 as compared to control group (fig. 1).

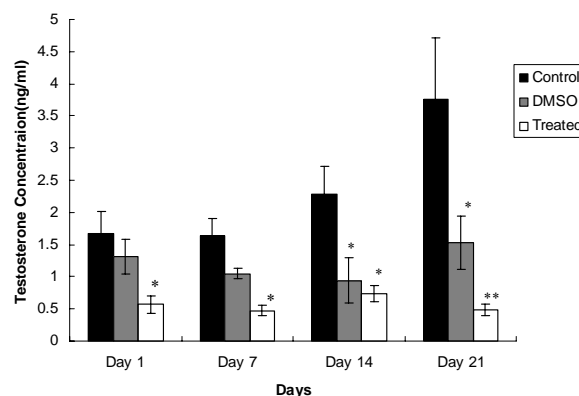


Fig. 1: Plasma testosterone concentrations in control (n=4), DMSO (n=4) and treated (n=4) groups at day 1, 7, 14 and 21.

* $P < 0.05$ vs control group, ** $P < 0.01$ vs control group

Morphometry

Testicular structure

Treatment of antithyroid drug 2,8-Dimercapto-6-hydroxypurine caused a highly significant decrease in tunica thickness of treated ($P < 0.0001$) and DMSO ($P < 0.001$) groups as compared to control group. Similarly seminiferous tubule epithelial height of both treated and DMSO group after 21st day of post-treatment showed a highly significant decrease ($P < 0.0001$ and $P < 0.001$ respectively) in mean tubular height as compared to control group. Testicular seminiferous tubule diameter of treated group also showed highly significant decrease ($P < 0.0001$) as compared to control group while a non-significant ($P > 0.05$) decrease was observed between DMSO and control group (table 1).

Cell count

After 21 day treatment of 2,8-Dimercapto-6-hydroxypurine the number of different cells per seminiferous tubule were counted. Number of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, Sertoli and Leydig cells showed a highly significant decrease in DMSO ($P<0.001$) and treated ($P<0.0001$) groups as compared to control group (table 2).

Nuclear Diameter

Nuclear diameter of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, leydig cells and sertoli cells decreased significantly in DMSO and treated groups as compared to control group table 3.

Histomorphology

The structural organization of antithyroid treated testes was markedly different as compared to control and DMSO groups. The 20 mg/kg intraperitoneal infusion of

anti-thyroid drug for 21 consecutive days caused significant alteration in the histological appearance of the testes in treated rats.

Histologically, transverse testicular section of anti-thyroid drug treated rats testes revealed that mostly the seminiferous tubules were distorted in shape. There was a significant decrease in the germinal epithelium height and seminiferous tubule diameter of the anti-thyroid treated rats (fig. 2 and table 1). A significant decrease in the cell population and diameter of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids were observed in anti-thyroid treated rat testes (fig. 3 and table 3). Testes of anti-thyroid drug treated rats showed marked decrease in the spermatogenic activity in the lumen of seminiferous tubule (fig. 4). The Leydig cell population and diameter decreased in interstitial spaces in anti-thyroid drug treated male rats (table 2). Distorted Sertoli cells were observed in seminiferous tubule and their number and diameter were decreased as compared to control and DMSO treated rats (fig. 3 and table 3).

Table 1: Effect of i.p antithyroid drug 2,8-Dimercapto-6-hydroxypurine administration (20 mg/kg) on tunica thickness, seminiferous tubule diameter, seminiferous tubule epithelial height in seminiferous tubule of treated (n=4) and DMSO (n=4) as compared to control (n=4), after 21 days.

Testicular Tissue	Control	DMSO	Treated
Tunica albuginea thickness (μm)	39.95 \pm 0.35	36.06 \pm 0.44**	31.90 \pm 0.55***
Seminiferous tubule diameter (μm)	247.20 \pm 2.28	246.03 \pm 1.47	233.21 \pm 1.73***
Seminiferous tubule epithelial height (μm)	53.08 \pm 0.31	43.25 \pm 0.26**	32.28 \pm 0.27***

*** $P<0.0001$ vs control, ** $P<0.001$ vs control

Table 2: Effect of i.p antithyroid drug 2,8-Dimercapto-6-hydroxypurine administration (20 mg/kg) on cell population of different cell type in seminiferous tubule of treated (n=4) and DMSO (n=4) as compared to control (n=4), after 21 days.

Cell count in Seminiferous Tubule	Control	DMSO	Treated
Spermatogonia	54.65 \pm 0.2	42.77 \pm 0.4**	36.76 \pm 0.2***
Primary Spermatocyte	48.96 \pm 0.4	37.05 \pm 0.3**	32.78 \pm 0.1***
Secondary Spermatocyte	47.15 \pm 0.3	32.38 \pm 0.3**	27.45 \pm 0.3***
Spermatid	28.18 \pm 0.3	24.05 \pm 0.2**	15.81 \pm 0.2***
Leydig Cell	8.98 \pm 0.1	7.08 \pm 0.2**	6.06 \pm 0.1***
Sertoli Cell	32.38 \pm 0.2	21.49 \pm 0.2**	14.37 \pm 0.3***

*** $P<0.0001$ vs control, ** $P<0.001$ vs control

Table 3: Effect of i.p antithyroid drug 2,8-Dimercapto-6-hydroxypurine administration (20mg/kg) on mean cell nuclear diameter of different cell type in seminiferous tubule of treated (n=4) and DMSO (n=4) as compared to control (n=4), after 21 days.

Nuclear Diameter (μm) of different Cell Type in Seminiferous Tubule	Control	DMSO	Treated
Spermatogonia	7.07 \pm 0.01	5.72 \pm 0.07**	5.25 \pm 0.03***
Primary Spermatocyte	5.55 \pm 0.07	5.22 \pm 0.03*	4.95 \pm 0.07**
Secondary Spermatocyte	4.86 \pm 0.10	4.76 \pm 0.10*	4.43 \pm 0.07**
Spermatid	4.56 \pm 0.06	4.20 \pm 0.05*	3.72 \pm 0.03**
Leydig Cell	5.17 \pm 0.03	4.82 \pm 0.05*	4.17 \pm 0.04**
Sertoli Cell	7.20 \pm 0.08	7.03 \pm 0.03	6.02 \pm 0.07**

* $P<0.01$, ** $P<0.001$, *** $P<0.0001$ vs control

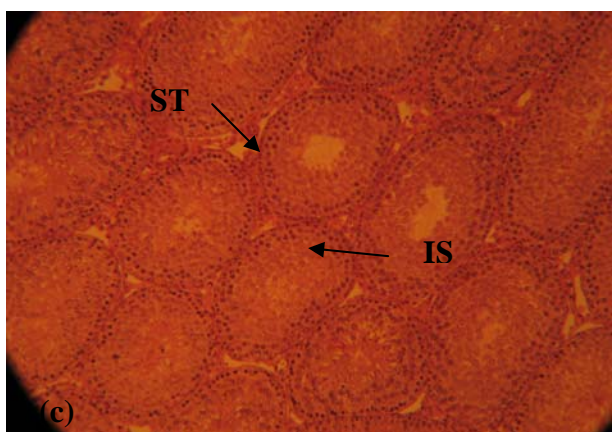
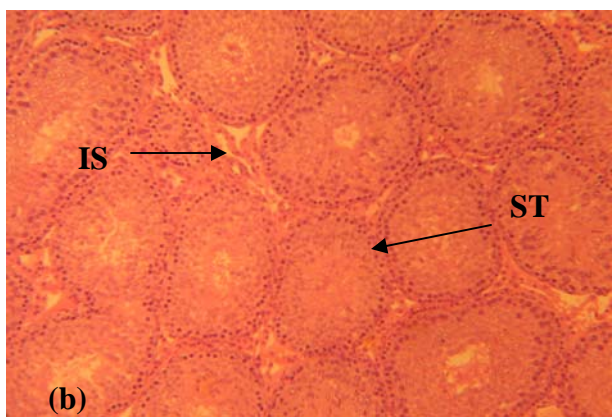
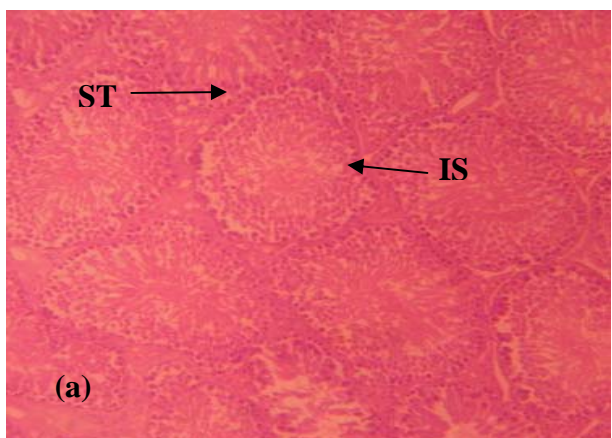


Fig. 2: Photomicrograph of T.S. of adult rat testes (20X) showing effect of 2,8-Dimercapto-6-hydroxypurine on seminiferous tubule arrangement after 21 days treatment (a) Control (b) Treated (c) DMSO; (ST) Seminiferous tubule, (IS) Interstitial space.

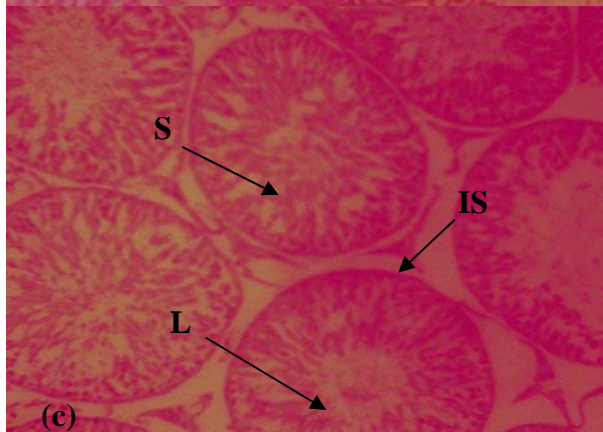
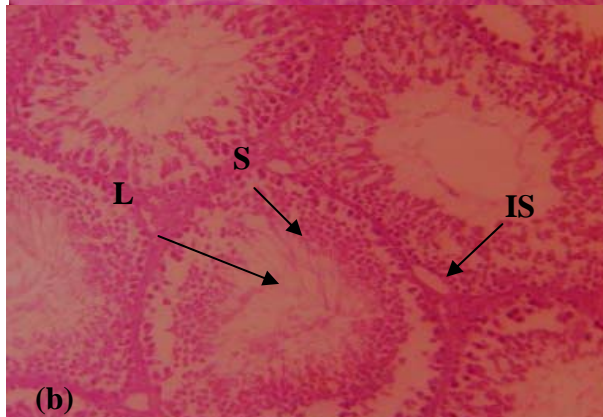
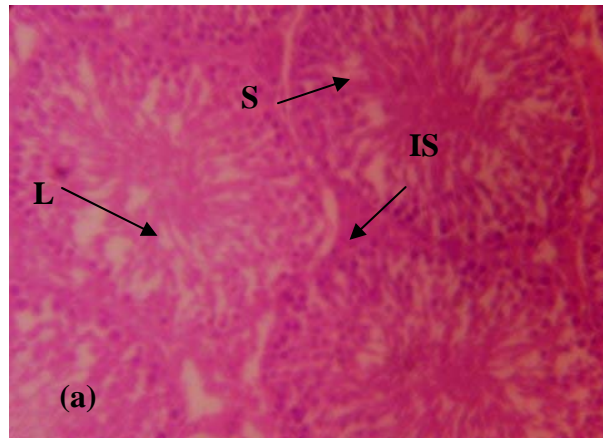


Fig. 3: Photomicrograph of T.S. of adult rat testes (40X) showing effect of 2,8-Dimercapto-6-hydroxypurine on spermatogenesis after 21 days treatment. (a) Control (b) Treated (c) DMSO; (L) Lumen, (S) Spermatozoa, (IS) Interstitial space.

Arrested spermatogenesis was observed in anti-thyroid drug treated testes and showed no proliferation of spermatogenic activity (fig. 3).

DISCUSSION

The present study was designed to investigate the possible role of derivative of antithyroid drug PTU (2,8-

Dimercapto-6-hydroxypurine) on morphometric studies of rats and reproductive axis of adult male rats. In this study the rats were administered with intraperitoneal infusion of 2,8-Dimercapto-6-hydroxypurine 20 mg/kg for 21 days when the rats were 60 days old and the rats were dissected on the 22nd day when the rats were 82 days old and it is in accordance that the sexual development in the male rats begins (Clermont *et al.*, 1957). The effect of

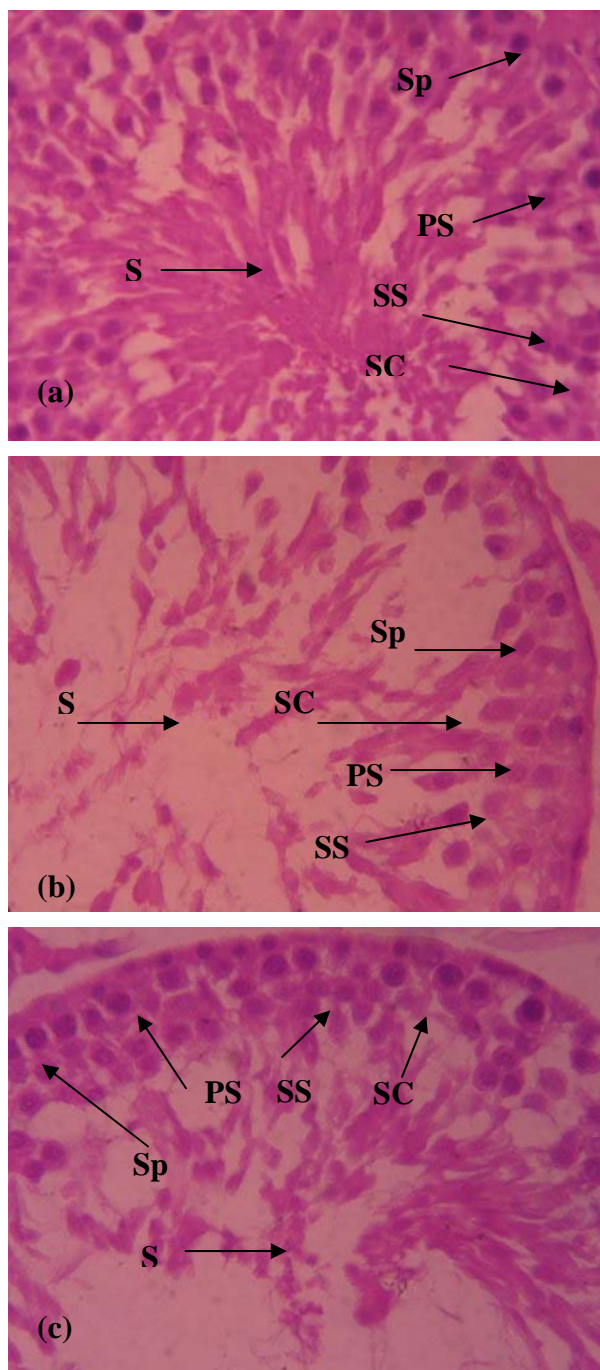


Fig. 4: Photomicrograph of T.S. of adult rat testes (100X) showing effect of 2,8-Dimercapto-6-hydroxypurine on spermatogenesis in single seminiferous tubule after 21 days treatment. (a) Control (b) Treated (c) DMSO; (Sp) Spermatogonia, (PS) Primary spermatocyte, (SS) Secondary spermatocyte, (SC) Sertoli cell, (S) Spermatozoa.

i.p administration of anti-thyroid drug 2,8-Dimercapto-6-hydroxypurine on mean testicular cell population and diameter in adult male rats. Similarly, significant decrease in testicular cell population and diameter in DMSO

treated rats was also observed. The seminiferous tubule depicted a state of arrested spermatogenesis. These findings are in accordance with the previous studies that arrested spermatogenesis was observed with tubules having few spermatocytes in neonatal hypothyroid rats (Cristovao *et al.*, 2002). However changes in the level or pattern of secretion of hypothalamic and pituitary hormones that directly or indirectly regulate the testes (e.g., thyroid stimulating hormone, thyrotropin-releasing hormone, gonadotrophin releasing hormone GnRH, follicle stimulating hormone FSH, lutenizing hormone LH) and/or changes in the testes may be involved (Cooke *et al.*, 1992). In the testes, exposure to 12 ppm PTU or MMI delayed spermatogenesis was observed with increasing number of apoptotic spermatocytes (Shibutani *et al.*, 2009).

Anti-thyroid drug treatment caused a significant decrease in Sertoli cell population and also in diameter in treated male rats. Similar findings of reduction of Sertoli cell population and diameter were observed also in DMSO treated testicular tissue in male rats. These results are in contrast to the previous findings that Sertoli cell number is increased following neonatal hypothyroidism (Jansen *et al.*, 2007). Delayed appearance of the tubular lumen and cytoplasmic lipids in the Sertoli cell may be the possible reason of retarded Sertoli cell development. Consistent with this possibility it is reported that early postnatal, but not prenatal, hypothyroidism retards Sertoli and germ cell maturation (Francavilla *et al.*, 1991). In this study a significant decrease in the Leydig cell number and diameter. Similar effects were observed in DMSO treated male rats. It is accordance with the previous findings of histological studies that hypertrophy of fetal Leydig cells and the appearance of adult Leydig cells in neonatal rat testes were prevented by hypothyroidism (Cristovao *et al.*, 2002). Testicular tunica thickness, seminiferous tubule diameter and seminiferous epithelial height were highly significantly reduced in the anti-thyroid treated rat testicular tissue. Similarly tunica thickness and seminiferous tubule epithelial of DMSO treated rat testes showed a significant reduction but a non-significant decrease was observed in seminiferous tubule diameter of DMSO treated rat testicular tissue in contrast to the control group rat testicular tissue. These findings correlate with the results that neonatal MMI methimazole treatment markedly inhibits Sertoli cell and development of germ cells and the testes as a whole. Neonatal MMI methimazole treatment significantly decreases testes weight, seminiferous tubule diameter and number of germ cell per tubule (Cooke *et al.*, 1993). Higher dose of PTU (0.5%) caused less pronounced effects on testes development. Lower dose (0.1%) had very low anti-thyroid effect however the higher dose (0.5%) induced severe hypothyroidism so it may be concluded that higher dose of PTU would be more beneficial (Knowlton *et al.*, 1999). Thus the decrease in the tunica thickness,

seminiferous tubule diameter and epithelial height may be due to administration of high dose of 2,8-Dimercapto-6-hydroxypurine to adult male rats.

The present study exhibits that high i.p. administration of anti-thyroid drug for 21 days significantly reduced the plasma testosterone levels on day 1, 7, 14 and 21. Similarly, a decrease in plasma testosterone level was also observed in DMSO treated rats at day 14 and 21. In the previous findings administration of PTU resulted in dose dependant inhibition of basal and human chronic gonadotrophin hCG, as well forskolin stimulated testosterone release by monkey testicular interstitial cells. PTU also diminished the stimulatory effects induced by androstenedione. These results suggest that PTU inhibits testosterone secretion via a mechanism independent of secretion of T4 and LH in primates. The inhibitory mechanism of PTU on testosterone production involves a decreased activity of 17 β -hydroxysteroid dehydrogenase (17 β -HSD) and post c-AMP pathways (Chiao *et al.*, 2000). PTU also inhibits testosterone release by acting directly on testis. Thyroidectomy and PTU induced hypothyroidism resulted in decrease in serum testosterone level. The decrease in testosterone production in response to hypothyroidism was attributed to decreased level of thyroid hormones in serum. PTU may directly regulate steroidogenesis in rodents. In an *in vitro* study PTU at pharmacological dosage level diminish basal and evoked testosterone secretion in rat testes (Chiao *et al.*, 2000).

In present study it may be concluded that anti-thyroid drug 2,8-Dimercapto-6-hydroxypurine is a negative modulator of reproductive axis and has anti-proliferative effect on mean testicular cell population and diameter. It exerts hypotrophic effects on testicular tissues and thus decrease in sperm production. It also decreases the plasma testosterone levels of the treated rats either by directly acting on the testes or by acting on the Leydig cells. However the exact mechanism is not known and this study needs further to be elucidated.

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