

Effect of green tea (*Camellia sinensis* L.) extract on morphological and functional changes in adult male gonads of albino rats

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Green tea, prepared from the steamed and dried leaves of the shrub *Camellia sinensis*, is known for its antioxidant and anti-carcinogenic effects. However, its effects on male gonadal functions have not been explored adequately and the present investigation has been undertaken to evaluate the effect of green tea extract on gonads of adult male albino rats. Results of *in vivo* studies showed that green tea extract (GTE) at mild (1.25 g%, \equiv 5 cups of tea/day), moderate (2.5 g%, \equiv 10 cups of tea/day) and high (5.0 g%, \equiv 20 cups of tea/day) doses, for a period of 26 days, altered morphology and histology of testis and accessory sex organs. A significant dose-dependent decrease in the sperm counts, inhibited activities of testicular Δ^5 3 β - and 17 β -hydroxysteroid dehydrogenase (Δ^5 -3 β -HSD and 17 β -HSD respectively) and decreased serum testosterone level were noticed. Significant increase in serum LH level was observed after moderate and high doses; serum FSH level also increased but not significantly. Histopathological examination showed inhibition of spermatogenesis evidenced by preferential loss of matured and elongated spermatids. Results of this study showed that GTE at relatively high dose may cause impairment of both the morphological and normal functional status of testis in rodents and thus its consumption at relatively high doses raises concern on male reproductive function in spite of its other beneficial effects.

Keywords: FSH, Green tea, LH, Sperm count, Testicular Δ^5 -3 β -HSD, Testicular 17 β -HSD, Testosterone

Plants are used globally as therapeutic agents since ancient times¹. Several plants are reported to enhance reproductive process and some are known to hamper such functions. Neem (*Azadirachta indica*)^{2,3} and Tulsi (*Ocimum sanctum*)⁴ are antifertility agents, while after ginger (*Zingiber officinale*)⁵ administration sperms are accumulated in the lumen of seminiferous tubules. Similarly, green tea extract has been used in traditional Chinese medicine for centuries to treat and prevent chronic diseases⁶. Green tea (*Camellia sinensis* L.) is prepared from the steamed and dried leaves of an evergreen shrub native to eastern Asia. It is consumed mainly in Japan, China and other Asian Countries including India⁷. Green tea, the minimally fermented (oxidized) product of the tea leaf, may show certain health benefits. In addition to their traditional use for making tea, the leaves of *Camellia sinensis* L. are also industrially processed. Many natural substances have been identified in green

tea; green tea components theanine and catechins have neuroprotective effects^{8,9}. It has significant role in cancer prevention. Green tea catechins have been shown to inhibit tumor cell proliferation and promote destruction of leukemia cells¹⁰ and breast cancer cells^{11,12}. Green tea was shown to decrease the risk of developing ovarian cancer¹³, and *in vitro* studies showed inhibition of proliferation of cervical¹⁴ and prostate¹⁵ cancer, head, neck¹² and pancreatic carcinoma cells¹⁶. Antioxidant action of phenol rich tea extracts reduced the ability of humans to utilize dietary iron. It has been suggested that excessive intake of tea should be avoided by people who are prone to anemia¹⁷. Epigallocatechin 3-galate (EGCG) inhibits type I 5 α reductase activity *in vitro*, which is partially responsible for conversion of testosterone to dihydrotestosterone¹⁸. Green tea was shown to be an aromatase inhibitor in rat; a causative factor for an increase in testosterone level^{19,20}. It has been also reported that there was a reduction in plasma testosterone level by green tea epigallocatechin galate. Goitrogenic/antithyroidal effect of GTE, in relatively high doses, has been reported both *in vivo* and *in vitro*²¹ and the role of thyroid hormone on the growth,

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and normal functioning of male gonads are well documented²². The present study was undertaken to evaluate the changes in testicular functions induced by GTE. A dose or concentration-dependent effects of GTE on testicular steroidogenic enzymes such as Δ^5 - 3β -hydroxysteroid dehydrogenase (HSD) and 17β -HSD, serum testosterone, LH, FSH levels, epididymal sperm count and histopathology of testicle were examined *in vivo*.

Materials and Methods

Animals—Adult (90±10 days) male albino rats (32) (*Rattus norvegicus L.*) of Sprague Dawley strain weighing 200±10 g were used in the present study. Animals were maintained as per national guidelines and protocols, approved by the Institutional Animal Ethics Committee (IAEC). Animals were housed in clean polypropylene cages and maintained in a controlled environment (temperature 22±2°C and relative humidity 40-60%) in an animal house under a photoperiod of 12 h of light and 12 h of darkness. Animals were fed on standardized normal diet (20% protein) which consisted of 70% wheat, 20% Bengal gram, 5% fish meal powder, 4% dry yeast powder, 0.75% refined til oil and 0.25% shark liver oil and water *ad libitum*²³.

Green tea extracts (GTE) preparation—Green tea was collected from Institute of Himalayan Bioresource Technology (IHBT), CSIR, Palampur, India. Composition of green tea was epicatechin (EC) 1.55%, EGCG epigallo catechin gallate (EGCG) 9.00%, ECG epicatechin gallate (ECG) 4.8%, EGC epigallo catechin (EGC) 5.0%, caffeine- 2.38%, as mentioned by manufacturer. Aqueous extract of green tea was done following the method of Wei *et al.*²⁴. To study the effect of GTE on male reproduction, the doses used have been selected based on the studies conducted earlier^{21,25}. Briefly, 2.5 g green tea was added to 100 ml of boiling water and was steeped for 15 min. The infusion was cooled to room temperature and then filtered. Tea leaves were extracted a second time with 100 ml of boiling water and filtered, and two filtrates were combined to obtain a 1.25% tea aqueous extract (1.25% tea leaf/100 ml water). Similar procedure was performed with 5 g green tea and 10 g green tea to prepare 2.5% and 5.0% aqueous green tea extract respectively (GTE). All the three GTE were fed orally to animals at a dose of 1 ml/ 100 g body wt.

Animal treatment—Rats were equally divided into 4 groups according to the dose of treatment. Each

group consisted of 8 animals. Animals in Group I were treated orally with sterile distilled water, 1 ml/ 100 g body wt/day, for 26 days as vehicle and considered as control. Animals in Group II were treated orally with 1.25% (w/v) green tea extract (prepared on the day of treatment) for 26 days at a dose of 1 ml/100 g body wt/ day. Animals in Group III and Group IV were treated orally with 2.5% (w/v) green tea extract and 5.0% (w/v) green tea extract respectively (prepared on the day of treatment) for 26 days at a dose of 1 ml/100 g body wt/ day. Treatment schedule was selected to determine effect of green tea extract on two seminiferous cycles and duration of one seminiferous cycle is 13.2 days in albino rats^{26,27}. Animals were sacrificed 24 h after the last treatment by cervical dislocation. Blood samples were collected from hepatic portal vein under very light ether anaesthesia. Serum samples were separated by centrifugation and stored at -20°C for different hormone assay.

Body and organ weight—Body weights of animals were recorded on the first day before treatment with GTE (initial) and the day of sacrifice (final). Testicles and accessory sex organs viz., seminal vesicles, cauda epididymis, coagulating gland were dissected out, trimmed off the attached tissues and weighed. Relative weight of organs was expressed per 100 g body weight. Left testis of each rat was used for biochemical estimation.

Histopathology of testis—Immediately after removal, the testis were fixed in Bouin's fluid and embedded in paraffin. Sections of 5 μ thickness were taken from the mid portion of each testis and stained with haematoxylin-eosin for examination under a light microscope. Quantitative analysis of spermatogenesis was carried out by counting the relative number of each variety of germ cells at stage VII of the seminiferous epithelium cycle, i.e. type-A spermatogonia (ASg), preleptotene spermatocytes (pLSc), mid pachytene spermatocytes (mPSc) and step 7 spermatids (7Sd), according to the method of Leblond and Clermont²⁸. The nuclei of different germ cells were counted from 20 round tubules of each rat. All the counts (crude counts) of the germ cells were corrected for differences in the nuclear diameter by the formula of Abercrombie²⁹: true count = (crude count×section thickness)/(section thickness - nuclear diameter of germ cell). The nuclear diameter of each variety of germ cell was measured with a Leitz micrometer. The possibility of variable tubular

shrinkage in the sections of green tea extract and vehicle treated groups were eliminated by the index of tubular shrinkage which was obtained from the average number of sertoli cell nuclei containing prominent nucleoli in the sections of the treated rats divided by that of the controls³⁰. Theoretically, each primary spermatocytes, after two successive reduction divisions, forms four spermatids. Therefore, the mPSc to 7Sd ratio should be 1:4 (ref. 31). The % of 7Sd degeneration was calculated from this ratio. Subtraction of the % of 7Sd degeneration from control group of rats showed the effective percentage of spermatids degeneration.

Sperm count—Sperm count was determined by counting in a haemocytometer following the method of Majumdar and Biswas³². Sperm samples were collected from the cauda epididymis. To minimize error, the count was repeated at least 5 times for each rat by different workers.

Measurement of serum testosterone—Serum testosterone was assayed using ELISA kit obtained from Dia Metra, S.R.L. Italy (Code No. DKO002). In this method serum sample (25 μ l) was taken in a microplate well and enzyme-testosterone conjugate was added, then the reactant was mixed. After the completion of the required incubation period (1 h at 37°C) the antibody bound enzyme testosterone conjugate was separated from the unbound enzyme testosterone conjugate by decantation. Activity of the enzyme present on the surface of the well is quantitated by the reaction with tetra methyl benzidine (TMB) substrate solution with 15 min incubation and finally by adding 0.15 M H₂SO₄ as stop solution. The absorbance was read against blanking well at 450 nm within 30 min in ELISA Reader (Merck). The sensitivity of the testosterone assay was 0.075 ng/ml and intra and inter-run precision had a coefficient of variation of 4.6% and 7.5% respectively.

Radioimmunoassay (RIA) of leutenizing hormone (LH), follicle stimulating hormone (FSH)—Serum levels of FSH and LH were assayed by RIA³³ using reagents supplied by Rat Pituitary Distribution and NIDDK (Bethesda, MD, USA). Carrier frees ¹²⁵I for hormone iodination was obtained from Bhabha Atomic Research Center (BARC), Mumbai, India. Pure rat FSH (NIDDK-r FSH-I-11) and LH (NIDDK-r LH-I-11) were iodinated using chloramine-T (Sigma Chemical Company, St. Louis, MO, USA) as the oxidizing agent following standard procedure²⁹. The

second antibody was goat anti-rabbit γ -globulin purchased from Indo-Medicine (Friendswood, TX, USA). The intra-assay variation for FSH and LH was 5.0 and 4.5%, respectively. All samples were run in one assay to avoid inter-assay variation.

*Determination of Δ^5 - β -hydroxysteroid dehydrogenase enzyme activity*³⁴—For measurement of Δ^5 - β -hydroxysteroid dehydrogenase (HSD) enzyme activity, tissues were homogenized, maintaining chilling conditions (4°C) in 20% spectroscopic-grade glycerol containing 5 mM of potassium phosphate and 1 mM of EDTA at a tissue concentration of 100 mg homogenizing mixture in Potter-Elvehjem glass homogenizer. This mixture was centrifuged at 10,000 g for 30 min at 4°C in a cold centrifuge (REMI, C-30). The 200 μ l supernatant was mixed with 1 ml of 100 μ M sodium pyrophosphate buffer (pH 8.9) and 20 μ l of 30 μ g 17 β -Estradiol. Δ^5 - β -HSD activity was measured after the addition of 1 ml of 0.5 μ M NAD (Nicotinamide adenine dinucleotide phosphate) to the cuvette in a UV spectrophotometer (UV-1240 Shimadzu, Japan) at 340 nm against a blank without NAD. One unit of enzyme activity is equivalent to a change in absorbance of 0.001/min at 340 nm.

*Determination of 17 β -hydroxysteroid dehydrogenase enzyme activity*³⁵—To study testicular 17 β -hydroxysteroid dehydrogenase (HSD) enzyme activity, the same supernatant fluid (200 μ l) of homogenizing fluid (prepared as described above) was added with 1.5 ml of 440 μ M sodium pyrophosphate buffer (pH 8.9), 0.5 ml of bovine serum albumin (25 mg crystalline BSA) and 40 μ l of 0.3 μ M 17 β -Estradiol. 17 β -HSD activity was measured after the addition of 1 ml of 1.35 μ M NAD (Nicotinamide adenine dinucleotide phosphate) to the cuvette in a UV spectrophotometer (UV-1240 Shimadzu, Japan) at 340 nm against a blank without NAD. One unit of enzyme activity is equivalent to a change in absorbance of 0.001/min at 340 nm.

Determination of protein—Proteins were estimated biochemically by the method of Lowry³⁶ using bovine serum albumin (BSA) as the standard protein.

Statistical analysis—Results were expressed as mean \pm standard deviation. One-way analysis of variance (ANOVA) test was first carried out to test for any differences between the mean values of all groups. If difference between groups were established, the values of the treated groups were compared with those of the control group by a multiple comparison *t*-test. A value of $P < 0.05$ was considered as statistically significant³⁷.

Results

Body weight of control animals increased progressively throughout the period of treatment and showed a net body weight gain of +35.91% after 26 days. It was also observed that the animals treated with mild doses (1.25%) of GTE showed a net body weight gain of +35.86% after 26 days. However, the net bodyweight gain of the animals treated with moderate (2.5%) and high (5.0%) doses of GTE administration were less and were +25.86% and +21.30% respectively after the duration of the treatment (Table 1).

Relative weight of the testis, cauda epididymis and coagulating gland were significantly decreased after

GTE administration as compared to control group of animals (Table 2).

Epididymal sperm number was significantly decreased in both the moderate and high doses of GTE administered groups of animals after 26 days treatment as compared with that of the control rats (Table 3).

There was no significant difference in serum FSH level after mild (1.25%), moderate (2.5%) and high (5.0%) dose of GTE administration (for 26 days) when compared with control group of rats. However, serum LH level was significantly increased in a dose- and time-dependent manner in GTE animals (for 26 days) as compared to their respective control group (Table 4).

Testicular steroidogenic enzyme activities viz. Δ^5 - 3β HSD and 17β -HSD significantly decreased after moderate and high doses of GTE administration compared with control; the activities remained unchanged after mild dose of GTE administration in the respective group (Figs 1 and 2).

Serum testosterone level decreased significantly in a dose-dependent manner in GTE administered animals compared to control animals (Fig. 3).

Testicular section of control and mild GTE treated groups of animals showed no pathologic features. However, at a moderate dose for 26 days caused significant reduction in the number of spermatogonia

Table 1—Body weight (g) of rats treated with green tea extract (GTE) at different doses for 26 days
[Values are mean \pm SD of 8 animals]

Control	Initial weight	Final Weight
	200.50 \pm 8.26	272.50 \pm 16.69
(% Gain in body wt)		(+35.91%)
Mild dose of GTE (1.25%)	190.00 \pm 7.33	258.13 \pm 13.21
(% Gain in body wt)		(+35.86%)
Moderate dose of GTE (2.5%)	195.25 \pm 3.99	245.75 \pm 5.18
(% Gain in body wt)		(+25.86%)
High dose of GTE (5.0%)	199.00 \pm 8.14	241.38 \pm 9.02
(% Gain in body wt)		(+21.30%)

Table 2—Weight of testis and accessory sex organs treated with green tea extract (GTE) at different doses for 26 days
[Values are mean \pm SD of 8 animals]

	Control	Mild dose of GTE (1.25%)	Moderate dose of GTE (2.5%)	High dose of GTE (5.0%)
Testis ¹	1.20 \pm 0.16	1.20 \pm 0.16	1.04 \pm 0.08 ^a	0.97 \pm 0.13 ^a
Cauda epididymis ²	221.10 \pm 21.04	219.12 \pm 20.04	205.40 \pm 14.58 ^a	200.42 \pm 23.40 ^a
Coagulating gland ²	63.96 \pm 10.21	61.96 \pm 9.21	48.52 \pm 5.80 ^a	47.14 \pm 8.27 ^a

¹g/100 g body wt, ²mg/100 g body wt. *P* value: < 0.05; compared to ^acontrol.

Table 3—Green tea extract (GTE) induced alteration at different doses on sperm count and the relative number of germ cells per tubular cross-section at stage VII of the seminiferous epithelial cycle (Duration of treatment: 26 days)

	Control	Mild dose of GTE (1.25%)	Moderate dose of GTE (2.5%)	High dose of GTE (5.0%)
Sperm count ¹	112.72 \pm 5.23	110.38 \pm 2.84	89.28 \pm 1.18 ^a	78.40 \pm 1.09 ^a
ASg	0.74 \pm 0.21	0.64 \pm 0.13	0.36 \pm 0.24 ^a	0.22 \pm 0.32 ^a
pLSc	20.28 \pm 1.73	18.82 \pm 0.59	17.78 \pm 0.83 ^a	17.33 \pm 1.49 ^a
mPSc	20.30 \pm 1.36	18.30 \pm 0.76	18.83 \pm 0.54 ^a	19.78 \pm 1.67 ^a
7Sd	65.12 \pm 1.52	57.87 \pm 1.25	47.15 \pm 1.24 ^a	45.69 \pm 0.95 ^a
mPSc:7Sd	1:3.21	1:3.16	1:2.50	1:2.31
7Sd degeneration (%)	19.75	21.0	37.5	42.25
Effective 7Sd degeneration	0	0.2	14.7	18.7

¹million cells/cauda epididymis. *P* value: < 0.05; compared to ^acontrol.

A (ASg) and step 7 spermatid (7Sd) resulting in a more prominent spermatogenic arrest as compared with those of the controls; high dose of GTE exposure for the same duration produced further testicular lesions (Fig. 4).

Discussion

Administration of green tea extract (GTE) orally, at relatively moderate (2.5%) and high (5.0%) concentration at the dose of 1 ml/ 100g body weight daily for 26 days, reduced the net gain in body weight of treated animals in comparison to that of their respective control. Decrease in body weight of the experimental animals was reported earlier after treatment with green tea and green tea powder respectively^{20,38}. The possible action of green tea on reduction in body weight is due to inhibition of the Catechol-O-methyl transferase (COMT) enzyme by EGCG of the tea³⁹⁻⁴¹. It has been shown that thermogenesis and fat oxidation are stimulated by norepinephrine, the action of which is degraded by the

enzyme COMT⁴⁰. Therefore, the inhibition of COMT enzyme activity by EGCG reduces the body weight. However, no such changes in body weight were recorded after mild dose of GTE as used in this study.

Along with the decrease in body weight, a significant reduction in testicular weight was also found in GTE treated animals in a dose dependent manner. Weight of the testis is largely dependent on the mass of the differentiated spermatogenic cells. Hence a reduction in its weight might be due to the

Table 4—Green tea extract (GTE) induced alteration at different doses on serum LH and FSH levels (Duration of treatment: 26 days)

[Values are mean ± SD of 8 animals]

	Control	Mild dose of GTE (1.25%)	Moderate dose of GTE (2.5%)	High dose of GTE (5.0%)
LH ¹	4.2±0.18	5.2±0.14	8.1±0.14 ^a	9.3±0.21 ^a
FSH ¹	8.1±0.08	8.8±0.08	9.8±0.08	11.9±0.05

¹ng/ml serum. *P* value: < 0.05; compared to ^acontrol.

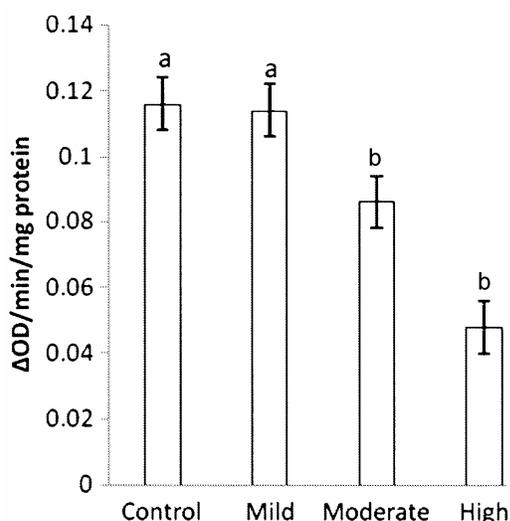


Fig. 1—Effect of green tea extract (GTE) at different doses on Δ⁵-β-HSD activity [Values are mean ± SD of 8 animals in each group. One-way analysis of variance (ANOVA) test followed by multiple comparison *t*-test. *P* value: < 0.05; compared to control. ^asignificant; ^bsignificant].

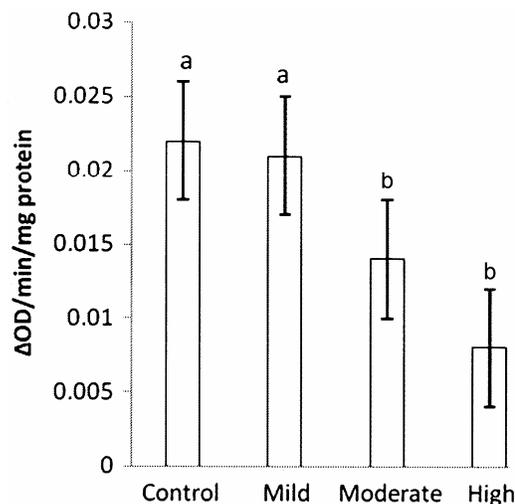


Fig. 2—Effect of green tea extract (GTE) at different doses on 17β-HSD activity [Values are mean ± SD of 8 animals in each group. One-way analysis of variance (ANOVA) test followed by multiple comparison *t*-test. *P* value: < 0.05; compared to controls. ^anot significant and ^bsignificant].

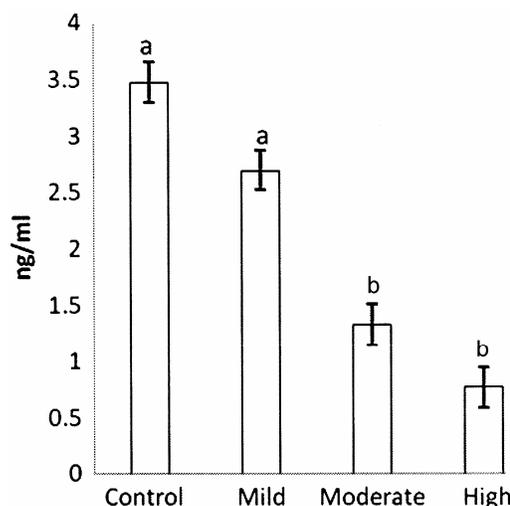


Fig. 3—Effect of green tea extract (GTE) at different doses on serum testosterone level [Values are mean ± SD of 8 animals in each group. One-way analysis of variance (ANOVA) test followed by multiple comparison *t*-test. *P* value: < 0.05; compared to controls. ^anot significant and ^bsignificant].

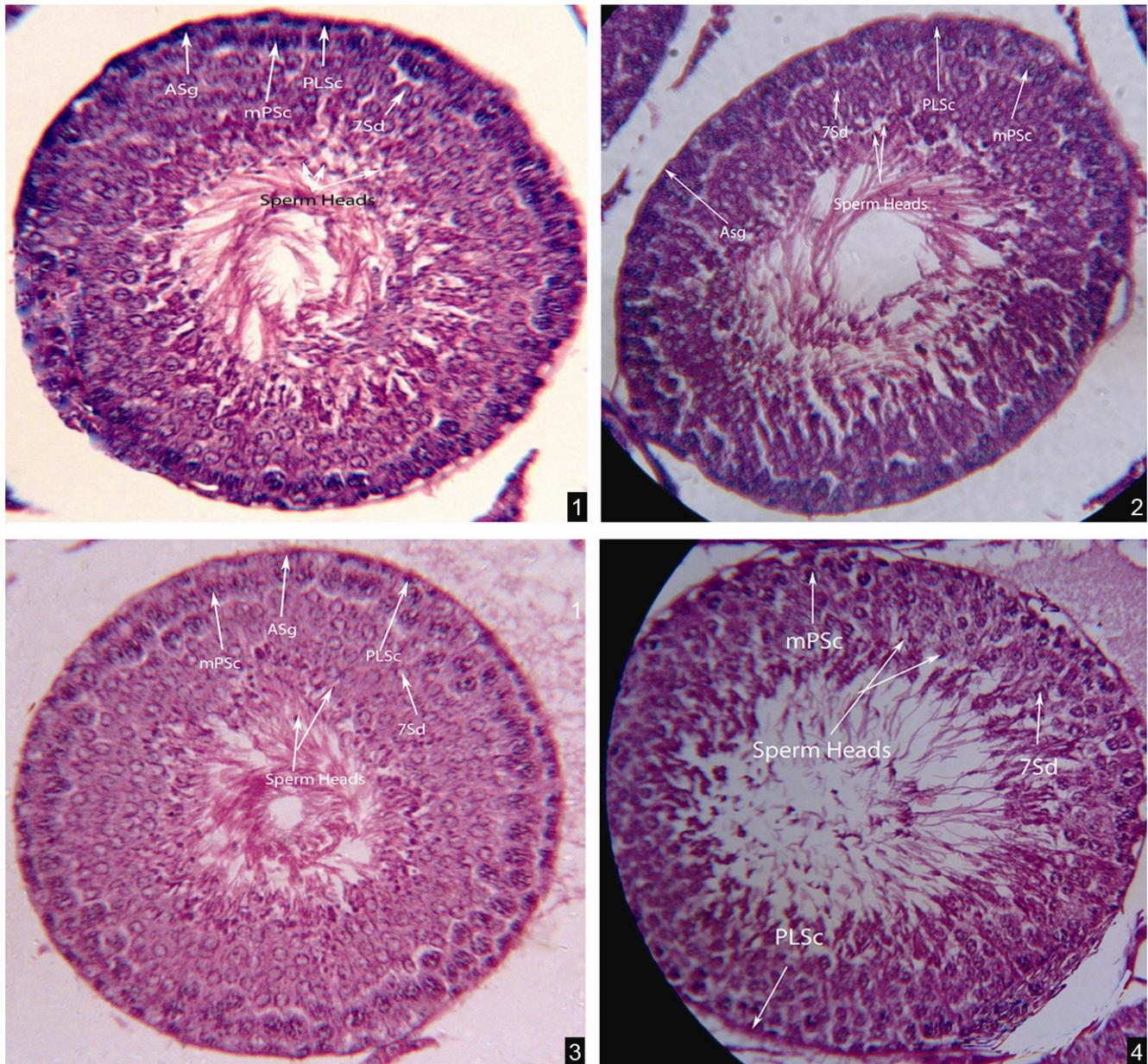


Fig. 4—Photomicrographs of seminiferous tubule (1)-control group showing more number of spermatogonia A (ASg), preleptotene (PLSc), midpachytene (mPSc) and round spermatids of stage 7 (7Sd) germ cells and more sperm heads; (2)-mild group showing less number of spermatogonia A (ASg), preleptotene (PLSc), midpachytene (mPSc) and round spermatids of stage 7 (7Sd) germ cells and less sperm heads than control; (3)-moderate group showing less number of spermatogonia A (ASg), preleptotene (PLSc), midpachytene (mPSc) and round spermatids of stage 7 (7Sd) germ cells and few sperm heads; (4)-high group showing lesser number of spermatogonia A (ASg), preleptotene (PLSc), midpachytene (mPSc) and round spermatids of stage 7 (7Sd) germ cells and few sperm heads, altered architecture of seminiferous tubule. H & E \times 40.

decreased number of germ cells and elongated spermatids⁴². Weight of accessory sex organs viz. coagulating gland and epididymis were also decreased in a dose dependent manner after GTE treatment. Weight loss of accessory sex organs corresponds with the decrease in serum testosterone concentration as observed in this study. It has been reported that

testosterone plays a major role in the maintenance of structural integrity and functional activities of the accessory sex organs⁴³.

As mentioned earlier, in the present study serum testosterone level was decreased in the GTE treated groups of animals as compared to their respective control. The decreased serum testosterone level as

observed might be due to the impaired synthesis of testosterone. Reduction in plasma testosterone level by green tea epigallocatechin gallet has been reported earlier¹⁵. On the contrary Satoh *et al.*¹⁹ found that green tea has an aromatase inhibitory activity which may be the causative factor for an increase testosterone level. In the present study, the activities of testicular steroidogenic enzymes $\Delta^5\beta$ HSD and 17β HSD were decreased significantly after GTE treatment in a dose dependent manner. $\Delta^5\beta$ HSD oxidizes pregnenolone to progesterone and 17β HSD produces testosterone from Δ^4 -androstenedione in the steroidogenic pathway. Therefore, decreased level of serum testosterone may be due to the decreased activities of these two steroidogenic enzymes. Kao *et al.*²⁰, also reported the decrease in serum testosterone level after exposure of catechin in green tea.

FSH, LH and testosterone are the useful markers in the diagnosis and management of male infertility⁴⁴. For the initiation of spermatogenesis and maturation of spermatozoa, FSH is necessary. In the fertile men, higher concentration of FSH is considered to be a reliable indicator of germinal cell damage, and has been shown to be associated with azoospermia and severe oligospermia⁴⁵. While the other gonadotropin, LH stimulates spermatogenesis indirectly through testosterone. Studies from rodent models suggest that gonadotropic hormones (both LH and FSH) facilitate the process of spermatogenesis by suppressing the proapoptotic signals and therefore, promote spermatogenic cell survival⁴⁶. In addition, the level of testosterone in serum regulates LH i.e., low level of testosterone stimulates more LH secretion through feedback mechanism. In GTE treated rats, decreased synthesis of testosterone results in the decrease of serum testosterone level which in turn elevates serum LH level through hypothalamo-pituitary feedback mechanism. Suppressed testosterone level also stimulates the secretion of more FSH, but not significantly as observed in this study.

It has been shown that in hypothyroidism, the functional status of gonads, indicating the levels of LH and FSH, levels of steroids, sperm count are decreased in adult male laboratory animals and humans^{47,48}. It has also been reported that oral administration of unfractionated green tea (mainly) and black tea administration at relatively high dose (5.0%) have the potential to induce hypothyroidism and thyroid gland enlargement in rats⁴⁹. Therefore, the altered thyroid functional status might have the role

through hypothalamo-pituitary-axis for enhancing the levels of LH and FSH (to an extent) in GTE treated animals.

Reduced epididymal sperm number in adult animals indicates that there must have certain interruption in the process of spermatogenesis as it represents the results of all the stages of meiosis, spermiogenesis and transition in the epididymis⁵⁰. In GTE exposed animals, the sperm count was reduced significantly. Marked reductions in the number of spermatogonia A (ASg) and step 7 spermatid (7Sd) were observed in the treated group as compared to the respective control. This indicates the impairment of spermatids maturation in the treated group. Theoretically, the ideal ratio of mPSc: 7Sd is 1:4 (ref. 31). In the present study, the ratios of mPSc: 7Sd were 1:3.0, 1:2.42 and 1:2.26 at mild, moderate and high doses (after 26 days of GTE treatment) respectively compared to respective control (1:3.01). According to Bansal and Devis⁵¹, alteration in this ratio also indicates spermatogenic impairment. In the present study, the percentage of spermatid degeneration (as calculated from above ratio) were found to be 40% and 42.5% in moderate and high doses of GTE respectively, which are highly significant.

Overall observations revealed marked damage in both the histoarchitecture and functional status of testis in GTE treated animals and the changes in the testis and accessory organs were dose dependent in nature. Whether the GTE induced testicular dysfunctions are the direct action of the GTE through the interference of the activities of steroidogenic enzymes on testis or through the impairment of thyroid gland activity or interference of both needs further investigation.

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