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Research Article

# GREEN TEA LEAF EXTRACT AS A HERBAL CASTRATIVE AGENT ON MORPHOLOGICAL AND FUNCTIONAL CHANGES IN ADULT MALE GONADS OF ALBINO RATS

**SHYAMAL KANTI DAS[1], SOUMENDRA NATH KARMAKAR[1], CHANDAN ROY[2]**

[1]Post Graduate Department Of Physiology, Krishnath College, Berhampore, Murshidabad, West Bengal, India, [2]Dept. of Physiology, Berhampore Girls' College, University of Kalyani, Atuhatpara (Near laxmi electric)
Email: iamchandan@rediffmail.com

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**ABSTRACT**

Experiment has been done to show the effect of Green Tea Leaf Extract (GTLE) on male reproductive system and also justify its effect to use GTLE as a castrative agent. Green Tea Leaf Extract was prepared according to the method used in Wei. H. *et al* (1999). The extract was given to the two different experimental animal groups with two different doses. After applying the doses in 26 consecutive days, it was found that the weight of the testis and epididymis was markedly decreased in highly treated group. The sperm count and its motility was also reduced drastically. But the alteration of enzymes like Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) was not significant. Result of this study showed that GTLE, relatively at high dose has its castrative activity on male reproductive system. Histological examination showed inhibition of spermatogenesis as evidence by disintegration of seminiferous tubules of testis.

**Keywords:** Green tea leaf extract, sperm count, Sperm motility, Histological changes, Castrative agent.

## INTRODUCTION

Population explosion is one of the burning problems in this century. This uncontrolled growth rate might be a threat to our nation in recent future. So, to survive against this unfavourable condition, it is a demand to the science to search for way out to overcome this problem. In a journey to search for contraceptive measures to birth control, some chemicals have been found till now, most of which are female contraceptive measures. Furthermore some physical methods are also available to control this growth rate. Among these physical methods, condom is highly acceptable measure for male. On the contrary surgical methods are also there to regulate the population explosion. These surgical methods are not always entertained due to its painfulness and also due to high expenditure. Besides these, there is also a plenty of post operative obligations which are hard to obey due to socio-economic problems. Taking these kind of circumstances into account there is an opportunity to think over some way which will be highly available and adaptable for all kind of people of our country and other developing countries. In this connection, a number of traditional Indian plant products have been used as herbal castrative agents for thousands of years. Several plants are reported to enhance reproductive ability and some are known to hamper such functions. Neem (Azadirachta indica)[1–2] and Tulsi (Ocimum sanctum)[3] are antifertility agents while after ginger (Zingiber officinale)[4]administration sperms are accumulated in the lumen of seminiferous tubule. It has been demonstrated earlier that Sarcostema acidum[5] stem extract exibit spermatogenic arrest in male rats without any side effects. Allium sativum[6] bulb extract has its spermicidal activities. It has also been demonstrated that methanolic pod extract of Albizzia lebbeck(L) Benth[7] has anti spermatogenic activity. Green tea components theanine and catechins have reproductive effects[8–9]. It has significant role in cancer prevention. Green tea catechin has been shown to inhibit tumor cell proliferation and promote destruction of leukemia cells[10] and breast cancer cells[11–12]. Green tea was also shown to decrease the risk of developing ovarian cancer[13]. It has been suggested that excessive intake of tea should have been avoided by those people who are prone to anaemia[14]. It has been also reported that there was a reduction in plasma testosterone level by epigallocatechin gallate present in green tea[15].It has been demonstrated earlier on that green tea leaf extract has significant role in decrease in testosterone level as well as changes in morphological character of testis[16]. The present study was undertaken to evaluate the changes in testicular functions induced by Green Tea Leaf Extract (GTLE) as well as to evaluate its castrative effect.

## MATERIALS AND METHODS

Adult (90±10 days) male albino rats of Wistar strain weighing 120-140 gm were taken for this experiment. Animals were maintained as per National guidelines and protocols. Animals were housed in clean polypropylene cages and were maintained in a controlled environmental temperature (22±2c) in an animal house und er a photoperiod of 12 hours of light and 12 hours of darkness with free access to water. Animals were fed on standardized normal diet (20% protein) which consists of 70% wheat, 20% gram, 5% fish meal powder, 4 % dry yeast powder and 1% oil and water adlibitum.

### Preparation of green tea leaf exrtract

Aqueous extract of Green tea leaf was prepared following the method of Wei. H. *et al* (1999)[17]. To study the effect of Green tea leaf extract on male reproduction, the doses were selected based on the study conducted earlier (Chandra A.K *et al* 2010 and Sakamoto Y *et al* 2001)[18–19]. At first 5.0 gm green tea was added to 100 ml of boiling water and was steeped for 15 min. The fusion was cooled to room temperature and was filtered. Tea leaves was extracted a second time with 100 ml of boiling water and filtered. Two filtrates were then combined to obtain a 2.5% tea aqueous extract (2.5 gm tea leaves/100 ml of water).Similar procedure was performed with 10gms green tea to prepare 5.0% aqueous green tea extract. The extract was then ready for oral administration.

### Animal treatment

Rats were equally divided into three groups (n=12). Initial body weights of all the rats were recorded. Animals of group-I were treated as control group and sterile distilled water was given 1ml/100 gm of body weight. Animals in Group-II were given 2.5% aqueous green tea extract 1 ml/100gm of body weight to each animal and considered as moderate dose treated group. Animals in Group-III were given 5.0% green tea leaf extract, 1 ml/100 gm of body weight of experimental rats and considered as high dose treated group.

After completion of 26 days of treatment, final body weights of all the rats were taken and the rats were anaesthetized one after another with anaesthetic ether and blood was collected directly from hepatic portal vein and allowed to coagulate. Clear serum was collected and stored in 20°c for enzyme assay. Testis and e pididymis of each rat were dissected out and treamed off adipose tissues and weights were taken. One testis from each rat was processed for histology and 5µ thick sections were taken and stained with haematoxylene and eosin for further observation. After sacrifice, the

cauda portion was cut and it was kept in 1ml diluents at 37. After scattering it, sperms were dispersed into the fluid and it was taken for the count of sperm and its motility through the process of Majumder and Biswas[20].

Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT) were measured of all the control and experimental animals through the process of Kind and King[21]. Finally results were compared with the respective controls with the help of student's 't' test (Das 2005)[22] to generalize the effect of green tea leaf extract on reproductive system of male albino rat model.

## RESULTS

### Effect on body weight

Twenty six days after treatment of GTLE (in two different dilutions), it was found that in control group-I, body weight was increased by 30.61%. In group-II and group-III (treatment groups) body weight was increased by 21.92% and 17.25% respectively (Table: 1).

**Table 1: Comparison between initial and final body weight of rats treated with GTLE of different doses and respective controls. Values are mean (in gm), n=12 rats in each group.**

| | Control | Moderate | High dose |
|---|---|---|---|
| Initial | 138.00 | 136.00 | 130.25 |
| Final | 180.25 | 165.82 | 152.73 |

**Table 2: Effect of GTLE on SGPT and SGOT activity in male albino rats, Values are mean (IU/L), n=12 rats in each group.**

| | Control | Moderate | High dose |
|---|---|---|---|
| Sgpt | 55.94 | 55.63 | 55.47 |
| Sgot | 55.73 | 55.25 | 55.02 |

**Table 3.1: Comparison of testicular weight of control and GTLE treated rats, Values are mean (gm %), n=12 rats in each group.**

| Wt. of testis | Control | Moderate | High dose |
|---|---|---|---|
| | 0.94 | 0.86 | 0.80 |

**Table 3.2: Comparison of epididymal weight in control and GTLE treated rats, Values are mean (mg %). n=12 rats in each group.**

| Wt. of epididymis | Control | Moderate | High dose |
|---|---|---|---|
| | 96.55 | 86.85 | 84.36 |

**Table 4.1: Effect of GTLE on sperm count in control and treated groups, Values are mean (million/ml), n=12 rats in each group.**

| Sperm count | Control | Moderate | High dose |
|---|---|---|---|
| | 73.28 | 64.85 | 51.50 |

**Table 4.2: Effect of GTLE on sperm motility in control and treated groups, Values are mean ( %), n=12 rats in each group.**

| Sperm motility | Control | Moderate | High dose |
|---|---|---|---|
| | 61.12 | 54.72 | 47.34 |

### Effect of GTLE on SGPT and SGOT activities

Twenty six days after treatment of GTLE (in two different dilutions), there was no significant change in SGPT and SGOT activities when the value was compared with the control (Table 2).

### Effect of GTLE on testicular and epididymal weight

Twenty six days after treatment of GTLE (two different dilutions) there was highly significant change in testicular weight ($P<0.001$) (Table:3.1) and epididymal weight ($P<0.001$) (Table:3.2), when the values were compared with the control.

### Effect of GTLE on Sperm count and sperm motility

The number of sperms were counted according to the method of Majumder and Biswas[20]. It was found that number of sperms were decreased significantly ($P<0.001$) (Table: 4.1). Sperm motility was measured according to the same method applied for sperm count. It was also decreased significantly ($P<0.001$) (Table: 4.2) in GTLE treated groups.

### Effect of GTLE on Histology of Testis

A dose dependent disintegration of seminiferous tubules of testis along with decrease in somatic indices, along with increase in luminal areas associated with reduced accumulation of spermatozoa were noticed in GTLE treated testis when compared with control.

## DISCUSSION

From these observations it appears that GTLE is useful as a herbal castrative agent when applied in a specific dose. Oral administration of GTLE in different groups of animals produces reduction in net gain of body weight in group-II (2.5% GTLE) and group-III (5.0% GTLE) animals in comparison to that of their respective control. It has been reported earlier that the body weight was reduced after the treatment with green tea and green tea powder[15,23].This decreased body weight after application of green tea extract may be due to inhibition of the catechol-o-methyl transferase (COMT) enzyme by epigallocatechin gallate (EGCG) of the green tea[24–25].

This enzyme has been shown to degrade the effect of norepinephrine which can stimulate thermogenesis and fat oxidation[26]. Besides, decrease in body weight in high dose of tea extract significant reduction in testicular weight was also found in a dose dependent manner. Testicular weight generally depends on the mass of the spermatogenic cells. So it may be said that the reduction of testicular weight is due to the decreased number of spermatogenic cells[27].

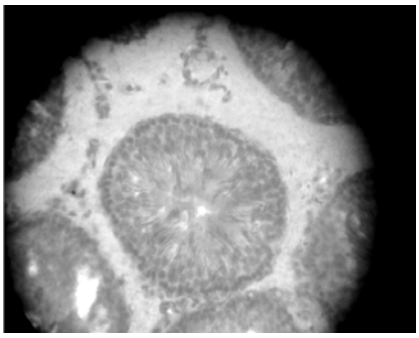


**Fig. 5.1: H/E stained section of control testis showing normal features (10 X 40 x)**

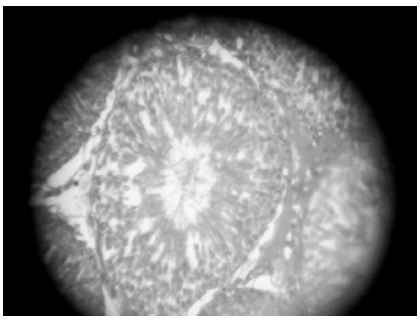
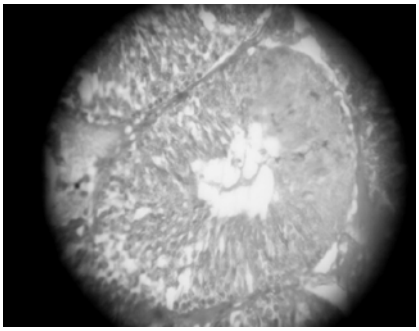


**Fig. 5.2: H/E Stained section of moderate dose (group-II) of GTLE treated testis (10 X 40 x).**



**Fig, 5.3: H/E Stained section of high dose (group-III) of GTLE treated testis (10 X 40x) showing an increase in luminal area, reduced spermatozoal mass and disorganized cellular orientation.**

Weight of the accessory sex organ- epididymis, was also decreased in a dose dependent manner after the application of green tea leaf extract. It may corresponds with the decrease in serum testosterone level because testosterone plays a major role in the maintainance of accessory sex organs[28].

With the application of green tea leaf extract in the present study, the sperm count and sperm motility were decreased in dose dependent manner in comparison with respective control. It might be due to the decrease in testosterone level with the application of green tea leaf extract[16] because testosterone and other gonadotropin like FSH and LH maintain the spermatogenesis or act as useful marker for male infertility[29].

In this present study, no change in the levels of SGPT and SGOT was observed in comparison with respective control. Analysis suggests that generally these two enzymes used as metabolic marker are inhibited due to failure in gastro-protective and repair mechanism leading to disrupted mucosal barrier[30]. So, in this present study it can be said that the metabolic activity may remain unchanged after oral administration of green tea leaf extract.

As a result of decreased testosterone level in response to the application of green tea extract[16], the sperm count may be reduced which was also reflected in the histological slide prepared from the moderate group and high treatment group of GTLE in comparison with control group. Vacuolization was seen in the middle of the testis, sperm heads were very rare at the centre and it was also scattered in the high treatment group. On the contrary, in control group, vacuolization was not so obvious and the sperm heads were distributed at the middle.

So, from the above study, it was revealed that marked damage in both the histoarchitecture and functional status of testis in GTLE treated animals and the changes in the testis and accessory organs were found in dose dependent manner. So GTLE may be a very important herbal castrative agent in near future.

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