

## GREEN TEA LEAF EXTRACT AS A HERBAL CASTRATIVE AGENT ON MALE ALBINO RAT

SHYAMAL KANTI DAS AND SOUMENDRA NATH KARMAKAR

*Post Graduate Department Of Physiology,  
Krishnath College, Berhampore, Murshidabad, W.B*

In this study Green Tea Leaf Extract (GTLE) is used as a herbal agent for application on animals for 26 consecutive days. This extract was prepared following the method of Wei *et al* (1999). The extract was administered to two different experimental animal groups with two different doses. After treatment, it was found that normal weight gains of all the animals were taken place but the percentage was slightly high in control animal group compared to two other experimental groups. Weight of the testis and epididymis were decreased after administration of GTLE compared to the control group of animals. SGPT and SGOT were not altered significantly after application of GTLE. Sperm count was reduced in experimental animal groups significantly in dose dependent manner. The testosterone level was also decreased in two treatment groups in dose dependent manner comparing to the animals of controlled groups.

It has become evident that increasing human population is a major problem of our nation. In near future it seems to be a threat and also a huge barrier for nation building. Keeping this problem in view, it is a little attempt to search for a new castrative agent which can be used as a easily available product and the method of execution would also be easy and within low cost. As the problem is increasing day by day, It has become compulsory to take step to stop the unfavourable condition of explosive growth rate of human population. To survive against this condition various contraceptive measures for birth control for male and female have been taken. There are some measures which are taken as drugs and others are physical methods. But these methods are not availed all the times due to uneasiness of uneducated people and sometimes for high cost for those people of below poverty level. In this connection, a number of traditional Indian plant products have been used as herbal castrative agents for many years. Several plants are reported to enhance reproductive ability and some are known to hamper such functions. Tulsi (*Oscimum sanctum*) (Kashinathan *et al*; 1972) and neem (*Azadirachta indica*) (Joshi *et al*; 1996 and Choudhury *et al*; 1990) are antifertility agents while after ginger (*Zingiber officinale*) (Khaki *et al*; 2009) administration causes accumulation of sperms in the lumen of seminiferous tubule. It has been demonstrated earlier that *Alium sativum* (Chakraborty *et al*; 2003) bulb extract has its spermicidal activities. *Sarcostema acidum* (Venma *et al*; 2002) stem extract exhibit spermatogenic arrest in male rats without any side effects. It has also been demonstrated that methanolic pod extract of *Albizzia lebbek* (L) Benth (Gupta *et al*; 2004) has anti-spermatogenic activity. Green tea components theanine and catechin have reproductive effects (Yokogoshi *et al*; 1998 and

\*Corresponding author. Soumendra Nath Karmakar, Dept. Of Physiology, Krishnath College, Berhampore, Murshidabad, W.B, Mob. 9732848327.

Kakuda *et al.*, 2007). It has significant role in cancer prevention. Green tea catechin has been shown to inhibit tumor cell proliferation and promote the destruction of leukemia cell (Smith *et al.*, 2001).

The main aim of this study is to investigate the efficacy of green tea leaf extract as a suitable agent for herbal castration without affecting the basic physiological parameters of the body.

### MATERIALS AND METHODS

Adult (90±10 days) male albino rats of Wistar strain weighing 120-140 gm were taken for this experiment. Animals were housed in clean polypropylene cages and were maintained in a controlled environmental temperature ( $22^{\circ} \pm 2^{\circ}\text{C}$ ) in an animal house under 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 ad libitum.

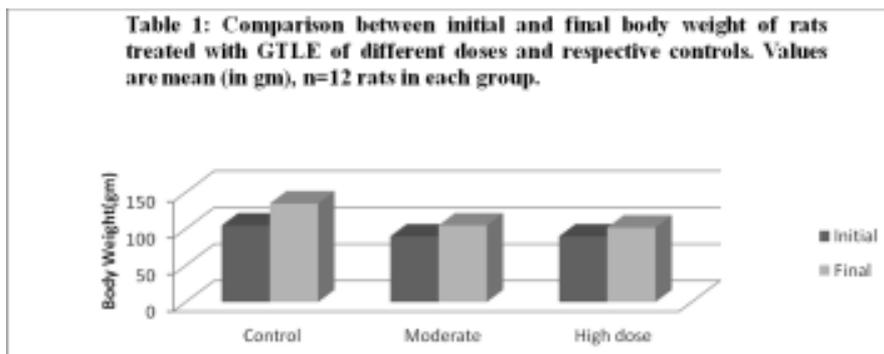
Aqueous extract of Green tea leaf was prepared following the method of Wei. *et al.*, (1999). To study the effect of Green tea leaf extract on male reproduction, the doses were selected based on the study conducted earlier (Chandra *et al.*; 2010; Sakamoto *et al.*, 2001). At first 5.0 gm green tea was 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.

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.

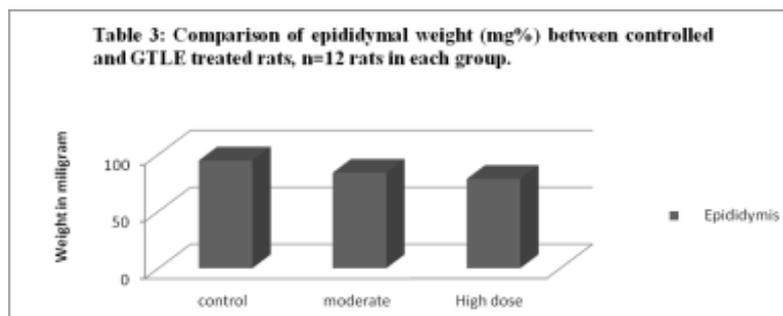
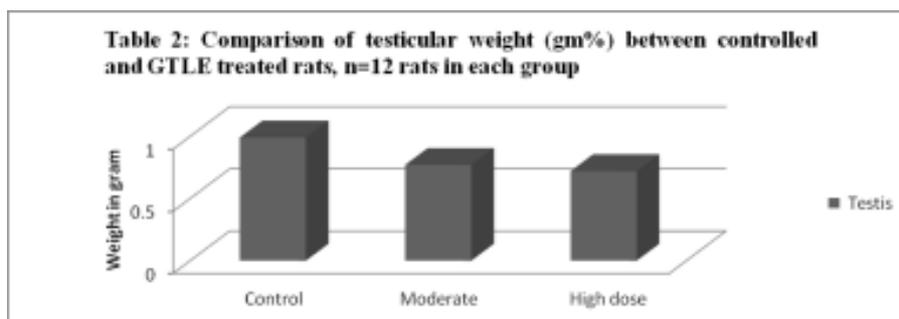
After completion of 26 days of treatment, final body weights of all the rats were taken and the rats were anaesthetized one after another with anaesthetic ether and blood was collected directly from hepatic portal vein and allowed to coagulate. Clear serum was collected and stored for enzyme assay. Testis and epididymis of each rat were dissected out and treated off adipose tissues and weights were taken. After sacrifice, the cauda portion was cut and it was kept in 1ml diluents at  $37^{\circ}\text{C}$ . After scattering it, sperms were dispersed into the fluid and it was taken for the count of sperm through the process of Majumder and Biswas (Majumder *et al.*, 1979). Hormonal level of serum like testosterone of all the animals were estimated with the help of ELISA method. Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT) were measured of all the control and experimental animals using the standard kits. Finally results were compared with the respective controls with the help of student's 't' test (Das, 2005) to generalize the effect of green tea leaf extract on reproductive system of male albino rat model.

**RESULTS**

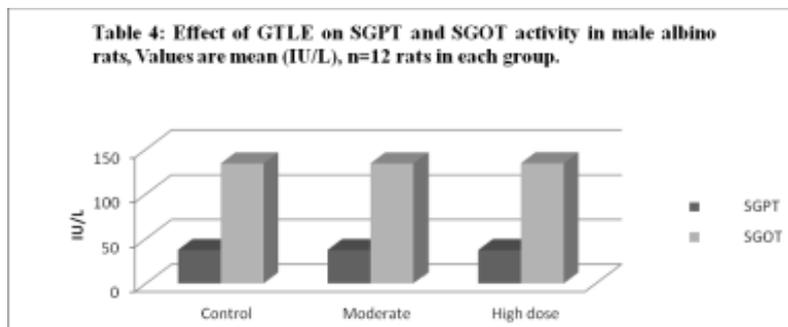
**Effect on body weight :** It has been observed from the present study that the net gain of body weight is decreased in moderate dose and high dose group accordingly in comparison with control group.



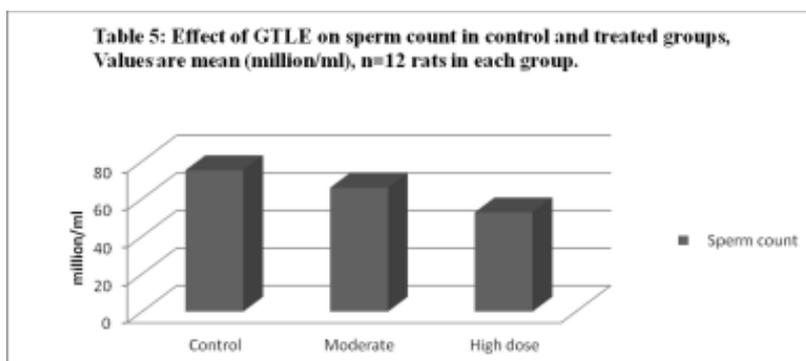
**Effect on testicular and epididymal weight :** Weight of testis and epididymis has been reduced significantly ( $p < 0.001$ ) after treatment of 26 consecutive days in high dose group.



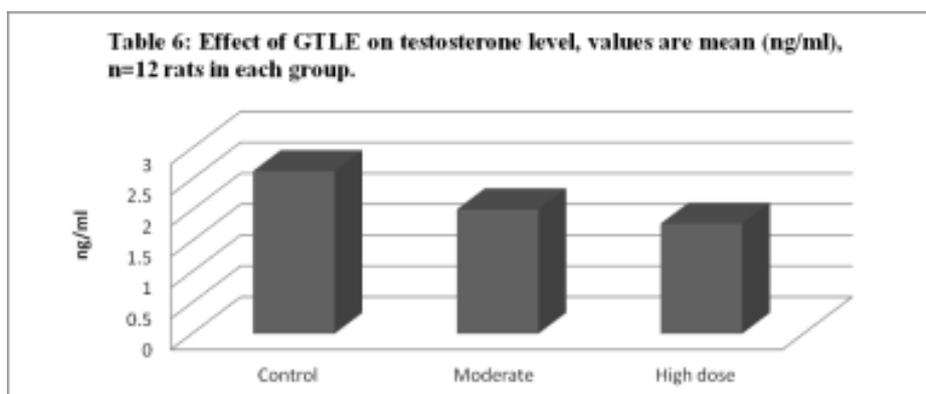
**Effect on SGPT and SGOT activities :** The enzymes system, SGPT and SGOT have not been changed significantly in the present study in both the moderate and high dose treated groups. These two enzymes system are considered as metabolic marker. So it means that GTLE has no influence on the metabolism of albino rat.



**Effect on sperm count :** Present observation clearly reflects on sperm count in moderate and high dose groups in dose dependent manner. In comparison with control group, sperm count of the treated groups have been reduced drastically ( $p<0.001$ ).



**Effect on serum testosterone level :** Administration of GTLE causes reduction in serum testosterone level after 26 days treatment. The change is significant ( $p<0.001$ ) in both moderate and high dose of treatment groups.



## DISCUSSION

After administration of Green Tea Leaf Extract in specific dose, it is clear that GTLE has its potency as a herbal castrative agent. After oral administration, GTLE produced reduction in net gain of body weight in moderate group and high dose treated group of animals in comparison to that of their respective control. Through various experiments earlier on, it has been shown that the body weight was reduced after the treatment of green tea and green tea powder (Sayana *et al.*, 2000). It has also been shown that the reduction of body weight after application of green tea extract may be due to inhibition of catechol-O-methyl transferase (COMT) enzyme by epigallocatechin gallate (EGCG) of the green tea (Chantre *et al.*, 2002). This enzyme is responsible to degrade the effect of nor epinephrine which can stimulate thermogenesis and responsible for oxidation of fat (Dulloo *et al.*, 2000).

High dose of green tea extract causes decreased testicular weight. Spermatogenic cell is the main factor for weight of the testis. So it may be said that, testicular weight loss is due to the decreased number of spermatogenic cells (Chapin *et al.*, 1997).

Epididymal weight was changed in dose dependent manner. It may be due to the decrease in serum testosterone level. Because testosterone plays a vital role in the maintenance of the weight of accessory sex organs (Moor *et al.*, 1930).

No significant changes were occurred regarding the plasma level of SGPT and SGOT. Analysis also depicts 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 (Dhikav *et al.*, 2003).

Treatment of the animals with GTLE in different doses, it was found that the sperm count was significantly reduced in compare to the control group. It may be due to the reduction in testosterone level (Choudhury *et al.*, 2011).

The present experiment clearly depicts that the decrease in serum testosterone level in GTLE treated groups in a dose dependent manner in comparison with their respective control. The decreased concentration of serum testosterone has also been reported earlier by green tea epigallocatechin gallate (Adani *et al.*, 2000). This reduced concentration of testosterone may be due to decreased activity of steroidogenic enzymes,  $\Delta 5$   $3\beta$  HSD and  $17\beta$  HSD (Chandra *et al.*, 2011).

So it may be concluded that oral administration of green tea leaf extract at a specific dose is effective and less expensive method for birth control. This may be utilized in animal birth control also.

## ACKNOWLEDGEMENTS

Authors are grateful to all respected teachers and other support stuffs of K.N College, Berhampore, Murshidabad, W.B.

## REFERENCES

Adani, V M, Ahmad N, Mukhtar H, (2000): Molecular targets for green tea in prostate cancer prevention. *J. Nutr.* **133**, 2417S.

- Chakraborty K, Pal S, Bhattacharya A J K (2003): Sperm immobilization activity of *Allium sativum* L. and other plants extracts. *Asian. J. Androl*, **5**, 131-135.
- Chandra A K, Choudhury S R, De N, Sarkar M (2011): Effect of green tea (*Camellia sinensis* L.) extract on morphological and functional changes in adult male gonads of albino rats. *Indian J. of Experimental. Biol.* **49**, 689-697.
- Chandra AK, De N (2010): Goitrogenic/antithyroidal potential of green tea extract in relation to catechin in rats. *Int. J. Biol. Res. Assoc.* **48**(8-9), 2304-2311.
- Chantre P, Lairon D (2002): Recent findings of green tea extract AR25 (Exolise) and its activity for the treatment of obesity. *Phytomedicine: Int. J. Phytotherapy. and Phytopharmacology.* **9**(1), 3-8.
- Chapin RE, Harris MW, Davis BJ, Ward SM, Wilson RE, Mauney MA, *et al.* (1997): The effects of perinatal/juvenile methoxychlor exposure on adult rat nervous, immune, and reproductive system function. *Fundamental and applied toxicology: Official. J Soc. of Toxicol.* **40**(1), 138-157.
- Choudhury D, Singh J, Singh B, Varma SK. J N N P (2990): Antifertility effects of leaf extracts of some plants in male rats. *J. Indian. Biol.* **28**, 714.
- Choudhury KANS, Roy De. Sarkar M (2011): Effect of green tea *Camelia sinensis* L extract on morphological and functional changes in adult male gonads of albino rats. *Indian J Experimental. Biol.* **49**, 689-697.
- Das D, Das A (2005): *Statistics in biology and psychology*, Academic publishers, Kolkata, 4th 117-126p.
- Dhikav V, Singh S, Pande S, Chawla A, Singh A J K (2003): Non-steroidal drug-induced gastrointestinal toxicity: Mechanisms and management. *J Acad Clin Med* **4**, 315-32.
- Dulloo AG, Seydoux J, Girardier L, Chantre P, Vandermader J. Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. *Int J. Association for the Study of Obesity*, **24**(2), 252-258
- Gupta RS, Kachhawa JBS, Chaudhary R (2004): Antifertility effects of methanolic pod extract of *Albizia lebbek* (L.) Benth in male rats. *Asian J Andrology*, **6**(2), 155-159.
- Joshi AR, Ahamed RN, Pathan KM, Manivannan B (1996): Effect of *Azadirachta indica* leaves on testis and its recovery in albino rats. *Indian J Exp Biol*, **34**(11), 1091-1094
- Kakuda T (2007): Neuroprotective effect of the green tea components theanine and catechins. *J Biol Pharm. Bull.* **25**, 1513.
- Kashinathan S, Basu S, Ramakrishnan SJL (1972): Antifertility effect of *Ocimum sanctum* L. *J Indian Biol.* **10**, 23.
- Khaki A, Fathiazad F, Nouri M, Ozanci C, Khaki AAJC (2009): The effect of ginger on spermatogenesis and sperm parameters of rat. *J. Indian. Med.* **7**, 7.
- Majumder GC, Biswas R (1979): Evidence for the occurrence of an ecto-(adenosine triphosphatase) in rat epididymal spermatozoa. *J The Biochemical* **183** (3), 737-743.
- Moor C, Gallagher T, Price (1930): DJRF, Rat prostate cytology as a testis hormone indicator and the prevention of castration changes by testis-extract injections. *J. Arch. Toxicol* **45**, 71.
- Sakamoto Y, Mikuriya H, Tayama K, Takahashi H, Nagasawa A, Yano N, *et al.* (2001): Goitrogenic effects of green tea extract catechins by dietary administration in rats. *J. Arch. Toxicol.* **75**(10), 591-596.
- Sayana K, Lin S, Oguni I, Zheng G. Effects of green tea on growth, food utilization and lipid metabolism in mice, *In Vivo. J. Arch. Toxicol.* **14**, 481.

GREEN TEA LEAF EXTRACT AS A HERBAL CASTRATIVE AGENT ON MALE ALBINO RAT

Smith D M, Dou Q P (2001): Green tea polyphenols epigallocatechin inhibits DNA replication and consequently induces leukemia cell apoptosis. *Int J. Mol. Med.* **7**,645.

Venma PK, Sharma A, Mathur A, Sharma P, Gupta RS, Joshi SC, *et al.* (2002) : Effect of *Sarcostemma acidum* stem extract on spermatogenesis in male albino rats. *Asian J. Andrology.* **4**(1), 43-47.

Wei H, Zhang X, Zhao JF, Wang ZY, Bickers D, Lebwohl M (1996): Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damage by aqueous extracts from green and black teas. *J. Free. Radic. Biol. Med.* **26** (11-12):1427-1435.

Yokogoshi H, Kobayashi M (1998): Hypotensive effect of gamma-glutamylmethanamide in spontaneously hypertensive rats. *J. Life. Sci.* **62** (12),1065-1068.