

HERBAL CASTRATION BY MEANS OF ORAL ADMINISTRATION OF GREEN TEA LEAF EXTRACT (*Camellia sinensis* L.) ON MALE REPRODUCTIVE SYSTEM

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ABSTRACT

Aqueous extract of green tea leaf, used in this experiment has shown its impact on male reproductive system. Various blood parameters have been changed and the structure of testis is altered after oral administration of Green Tea Leaf Extract (GTLE). The extract was prepared according to the method of Wei.H.et al (1999). The extract was given to two different experimental animal groups with two different doses during 26 consecutive days. After oral administration of GTLE, it was found that, the weight of the testis and epididymis as accessory sex organ were markedly reduced instead of normal weight gain of all the animals. The sperm count and motility were reduced for the treated groups as compared with control animal group. The enzymes like SGPT and SGOT were as usual and other blood

parameters like glucose and protein were also as usual comparing with controlled group. Cholesterol level in the serum was insignificantly altered in dose treated groups. Testosterone level was reduced in the treated groups. FSH and LH levels were also altered accordingly in treated groups. Histological examination showed inhibition of spermatogenesis as evidenced by disintegration of seminiferous tubules of testis. Result of this study showed that GTLE has potent castrative activity on male reproductive system in dose dependent manner.

KEYWORDS: Green tea leaf extract, castrative agent, epigallocatechin, sperm motility, steroidogenic enzyme.

INTRODUCTION

It has become evident that increasing human population is a major threat to our nation. In near future it seems to be danger and also a huge barrier for nation building. So awareness should be spreaded out alongwith some adaptable measures which can be within reach for all the people of various economic groups. It is the compulsory step to be taken to stop the unfavourable condition of explosive growth rate of human population. To survive against this condition various contraceptive measures for birth control of male and female have taken. There are some measures 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. Surgical methods are also there to fight against this problem but major people discard this method for its painfulness nature. Besides this there is also a plenty of post operative obligation which are hard to obey due to lack of awareness and knowledge. In a journey to search for way out to overcome this problem and to reduce the population growth rate, we are very much glad to have some herbal castrative agents which may be availed by all kind of economic groups of people. 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*)^[1] and neem (*Azadirachta indica*)^[2-3] are antifertility agents while after ginger (*Zingiber officinale*)^[4] administration causes accumulation of sperms in the lumen of seminiferous tubule. It has been demonstrated earlier that *Alium sativum*^[5] bulb extract has its spermicidal activities. *Sarcostema acidum*^[6] stem extract exhibit spermatogenic arrest in male rats without any side effects. It has also been demonstrated that methanolic pod extract of *Albizia lebbek* (L) Benth^[7] has anti spermatogenic activity. Green tea components theanine and catechin 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 the destruction of leukemia cell^[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 epigallo catechin 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 morphological and functional changes in testis as well as hormonal level by administration of green tea leaf extract.

MATERIALS & METHODS

Adult (90±10 days) male albino rats of Wistar strain 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±2°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.

Preparation of green tea leaf extract

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 were 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 for enzyme assay. Testis and epididymis of each rat were dissected out and freed of adipose tissues and weights were taken. One testis from each rat was processed for histology and 5µ thick sections were taken and stained with haematoxyline and eosin for further

observation. After sacrifice, the cauda portion was cut and it was kept in 1ml diluents at 37°C. 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 glucose and cholesterol were measured using the standard kits. The serum protein was estimated by Biuret method with a standard curve of BSA. Hormonal level like testosterone, FSH and LH in serum 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 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

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.

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 body wt.	103	92	93
Final body wt.	133	105	104

Weight of testis has been reduced significantly ($p < 0.01$) and epididymis was also significantly ($p < 0.001$) reduced after treatment of 26 consecutive days in high dose group.

Table 2: Comparison of testicular (gm%) and epididymal weights (mg%) between controlled and GTLE treated rats, values are mean \pm SEM, n=12 rats in each group.

	Control	Moderate	High dose
Testis	0.97 \pm 0.060	0.89 \pm 0.076	0.81 \pm 0.051
Epididymis	93.55 \pm 0.469	84.86 \pm 0.307	81.20 \pm 0.170

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 ($p < 0.001$).

Table 3: Effect of GTLE on sperm count in control and treated groups, Values are mean \pm SEM (million/ml), n=12 rats in each group.

	Control	Moderate	High dose
Sperm count	76.50 \pm 0.426	65.20 \pm 0.171	51.50 \pm 0.426

Sperm motility has also been severely reduced after 26 days consecutive treatment of GTLE in dose dependent manner ($p < 0.001$) in comparison with control group.

Table 4: Effect of GTLE on sperm motility in control and treated groups, Values are mean \pm SEM (%), n=12 rats in each group.

	Control	Moderate	High dose
Sperm motility	71.40 \pm 0.341	60.00 \pm 0.426	54.20 \pm 0.426

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.

Table 5: Effect of GTLE on SGPT and SGOT activity in male albino rats, Values are mean \pm SEM (IU/L), n=12 rats in each group.

	Control	Moderate	High dose
SGPT	37.25 \pm 0.176	37.30 \pm 0.151	37.20 \pm 0.134
SGOT	133.20 \pm 0.128	133.18 \pm 0.205	133.30 \pm 0.214

GTLE has no influence on serum glucose, total protein and total cholesterol in moderate and high dose treated groups when compared with control.

Table 6: Comparison of glucose and serum protein between controlled and treated groups, values are mean \pm SEM (gm/100 ml), n=12 rats in each group.

	Control	Moderate	High dose
Glucose	100.20 \pm 0.171	100.30 \pm 0.256	100.32 \pm 0.017
Total protein	8.30 \pm 0.128	8.38 \pm 0.051	8.40 \pm 0.171

GTLE has changed the serum cholesterol level in the animals of two dose treated groups but the influence of the drug is not significant.

Table 7: Effect of GTLE on serum cholesterol level, values are mean \pm SEM (mg/dl), n=12 rats in each group.

	Control	Moderate	High dose
Cholesterol	92.98 \pm 0.401	93.27 \pm 0.290	93.30 \pm 0.352

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.

Table 8: Effect of GTLE on testosterone level, values are mean \pm SEM (ng/ml), n=12 rats in each group.

	Control	Moderate	High dose
Testosterone	2.83 \pm 0.111	2.20 \pm 0.170	1.80 \pm 0.085

FSH and LH have also been altered in two treated groups accordingly but not significantly in case of LH.

Table 9: Effect of GTLE on FSH & LH, values are mean \pm SEM (mIU/ml), n=12 rats in each group.

	Control	Moderate	High dose
FSH	3.79 \pm 0.162	4.62 \pm 0.060	4.95 \pm 0.128
LH	4.88 \pm 0.298	5.05 \pm 0.128	5.97 \pm 0.145

Histological structure has been changed in both the doses in treated groups. Disintegration of seminiferous tubules of testis alongwith decrease in somatic indices occurs. Reduction in the accumulation of spermatozoa is also observed when compared with control.

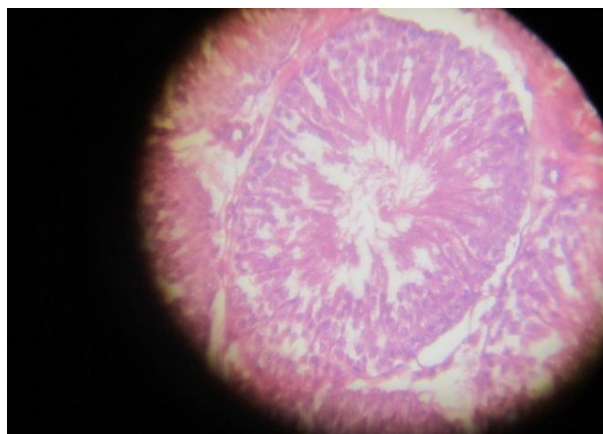


Fig. 9.1: H/E Stained section of moderate dose (group-II) of GTLE treated testis (10 X 40 x).

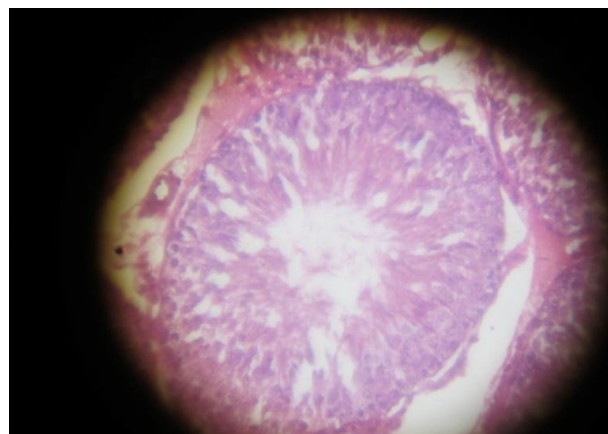


Fig. 9.2: H/E Stained section of high dose (group-III) of GTLE treated testis (10 X 40x) showing an increase in luminal area, reduced spermatozoal mass and disorganized cellular orientation.

DISCUSSION

From these observations, it has been found that GTLE is a potent herbal castrative agent when applied in a specific dose. When GTLE was administered orally in different groups of animals, it 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.^[15,23] 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.^[24-25] This enzyme is responsible to degrade the effect of nor epinephrine which can stimulate thermogenesis and responsible for oxidation of fat.^[26]

Besides body weight reduction, high dose of tea extract can cause significant reduction in testicular weight in dose dependent manner. Weight of testis generally depends on the mass of spermatogenic cells. So it may be said that, testicular weight loss is due to the decreased number of spermatogenic cells.^[27]

GTLE has its influence in alteration of accessory sex organ weight like epididymis in a 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.^[28]

After application of GTLE in various doses, the sperm count and sperm motility were decreased in both treated groups in comparison to their control. It may be due to the reduction in testosterone level.^[16] Because testosterone and other gonadotrophins like FSH and LH maintain the spermatogenesis and act as a useful marker for male infertility.^[29]

Present observation suggests that no change in the level of SGPT and SGOT occurs after treatment of animals with GTLE when compared with controlled animals. 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.^[30] So it can be concluded that the oral administration of GTLE does not hamper the metabolic activity.

Present experiment shows that glucose and total protein remain unchanged after treatment of animals with GTLE. These two components generally change their level in serum depending on oral ingestion of food stuffs but no alteration in their level in serum comparing the control suggest that there is no metabolic disruption after oral administration of GTLE. Though for bio-synthesis of androgen, cholesterol has major role but in present study its level is not altered significantly.

This experiment clearly shows 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.^[31] This reduced concentration of testosterone may be due to decreased activity of steroidogenic enzymes, Δ^5 3 β HSD and 17 β HSD.^[32] Kao et al^[33] also reported the decrease in serum testosterone level after exposure of catechin in green tea.

In present observation, GTLE treated groups of animals showed decreased synthesis of testosterone and also decreased serum level of this androgen which reflects in elevated level of serum LH through feedback mechanism^[34] insignificantly. Diminished level of testosterone also increases the FSH level significantly. Because all these hormones are treated as useful marker in management of male infertility.^[35]

Observation also suggests that, there are changes in histo architectures confirmed from the histological slide prepared from the moderate group and high treated group of GTLE. Comparing with control group, vacuolization in treated groups are also observed. The sperm heads are not properly distributed at right places. Heads of the sperms are scattered in case of high dose treated group.

CONCLUSION

So, from the above study it is revealed that, there are some changes in histo architecture and functional status of testis and also some impairment in accessory sex organ in dose dependent manner after oral administration of GTLE. So, it can be concluded that GTLE may be used as potent herbal castrative agent in near future.

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