IJPSR (2019), Volume 10, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 February 2019; received in revised form, 23 July 2019; accepted, 22 October 2019; published 01 November 2019

ASSESSMENT OF GREEN TEA EXTRACT ON TESTICULAR ACTIVITY IN STREPTOZOTOCIN-INDUCED DIABETIC SPRAGUE-DAWLEY RAT MODEL

Kamaldeep Singh [1], Savita Devi [2] and Pranay P. Pankaj [*3]

Galgotias University [1], Greater Noida - 201310, Uttar Pradesh, India.
Lovely Professional University [2], Phagwara - 144411, Punjab, India.
Department of Zoology [3], Nagaland University, Lumami - 798627, Nagaland, India.

Keywords:

Diabetes mellitus, Sexual abnormalities, Hypoglycemic agents, secondary complications, *Camellia sinensis*, Streptozotocin

Correspondence to Author:
Dr. Pranay Punj Pankaj

Assistant Professor,
Department of Zoology,
Nagaland University, Lumami - 798627, Nagaland, India.

E-mail: pranaypunj@gmail.com

ABSTRACT: Diabetes mellitus is a lasting metabolic disability regarding enhance etiology and permanent degenerating complications. Sexual abnormalities (Hypogonadism, infertility, *etc*.) are outfit a two of the discombobulate ingenious stringency of diabetes mellitus. Different hypoglycemic agents are used to control diabetes mellitus, but very few formulations are available to treat secondary complications. Results of the present study establish the anti-diabetic properties of *Camellia sinensis* and its impact on the male reproductive systems of the streptozotocin-induced diabetic male rat.

INTRODUCTION: Diabetes mellitus (DM) is a disease that deals with a chronic metabolic disorder involving carbohydrate, lipid, and protein by the occurrence of insulin scarce or disorder. Other conditions like arteriosclerosis (hardening and loss of elasticity of the wall of arteries), nephropathy (kidney damage), neuropathy (peripheral nerve dysfunction), and micro-angiopathy (thickening and weakening of capillary walls) can also be devoted through DM [1]. It is also associated with an increased incidence of cardiovascular disease, reduced life expectancy, giant morbidity due to specific diabetes-associated microvascular compli-cations and dwindled high-quality lifestyles [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(11).5063-68

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(11).5063-68

A link of DM with sexual dysfunction was perceived earlier in the 10th century when Avicenna reported that "collapse of sexual function" as a precise problem of DM [3]. Prolonged DM may lead to conditions such as impotence or erectile dysfunction, ejaculation disorder (premature or delayed ejaculation), and a decreased libido [4]. DM is responsible for biochemical variations and other pathological changes, which finally disturb male fertility.

The sexual dysfunctions such as spermatogenesis, retrograde ejaculation or erectile dysfunction occur, and these end up with decreased sexual appetite in diabetic individuals. DM results in altered pathways by multiple molecular mechanisms with dramatic consequences to male reproductive functions. Alteration in testicular cells is concerned maximally with glucose metabolism as glucose homeostasis plays a critical role in sub-fertility and fertility [5]. *Camellia sinensis* is commonly referred to as green tea, the World's widely consumed

beverages. Dried leaves of green tea (CS) contain active components like polyphenols (30%-36%). Flavanols present in a high amount which mostly known as catechins. The sub-category of catechins is epicatechin-3 gallate (ECG), epigallocatechin (EGC), epigallocatechin-3-gallate (EGCG) and epicatechin (EC).

CS has several natural properties in its active components which include cancer chemo-prevention, tumor cell suppressor, antiviral, antibacterial, anti-inflammatory [6], peritonitis [7] and antioxidant activities [8]. It retains suppressor effects on several enzymes like aromatase [9], angiotensin-converting enzyme [10] and thyroid peroxidase [11]. Green tea extract (GTE) ahead demonstrated to accommodate glucose metabolism divagate beneficially in progressive models of mark II diabetes mellitus.

An accomplice, EGCG, ameliorates cytokine-induced β-apartment execration *in-vitro* and prevents the obligation of ait hoard induced by sedative encircling go together wretched doses of streptozotocin *in-vivo* [12]. Most of the complications of DM have been studied widely, but sexual dysfunctions are still incompletely understood in the male with relation to plasma levels of testosterone, FSH and LH [13]. Modern societies concede a more in-depth look into the rate of fertility that revealed highly increased frequency of DM, which further has been correlated with falling fertility and birth rate [1].

Considering the therapeutic potential of CS, the direct of the tangible dissection is to analyze the point of CS extract on general physiology, fasting blood glucose level, protein, albumin, globulin level, lipid profile, and testosterone hormone in the streptozotocin-induced diabetic male SD rat model.

MATERIALS AND METHODS:

Plant Material: The green tea extract (GTE) material was procured from AM Labs, New Delhi, India. General specification of green tea extract powder (98% polyphenols/40% EGCG) is as follows:

Composition Specification: Total polyphenols (UV): min 98%; total catechins (HPLC): min 70%; content EGCG (HPLC): min 40%; content caffeine (HPLC): max 5%.

Physical Property:

Solubility: water-soluble; particle size: 100% through mesh size 60; loss on drying: max 5%; storage: stored in a cool, dry place, avoiding sunlight directly; shelf life: At least three years when stored properly.

Animals: Male Sprague-Dawley rats of weight (220-320 gm) and age three months, were obtained from NIPER, Mohali, Punjab. Rats were grouped into four groups of six animals and kept in steel cages; each cage consists not more than three rats in an air-conditioned room (22 ± 3°C, 55 ± 5% humidity and a 12-h light/dark cycle). A healthy diet and water *ad libitum* were given for feeding animals.

Following the rules of the Institutional Animal Care Committee (LPU/LSPS/IAEC/CPCSEA/ Meeting No 5, Jan 2015, Protocol No. 7) and the principles outlined in the declaration of Helsinki, all experimental processes were carried out.

Drugs and Chemicals: Streptozotocin was procured from Spectrochem, India. The assay kits were obtained from modern surgical house C-38, and surgical complex Basti Bawa Khel, Jalandhar, Punjab. Other analytical chemicals and biochemical reagents were used.

Induction of Diabetes: Streptozotocin dose was prepared by dissolving in normal saline at room temperature and placed the solution over the ice pack. It was always prepared freshly for immediate use and injected by intraperitoneal routes, in overnight fasted male rats. The doses were determined according to the bodyweight of animals [14].

Experimental Design: The experimental rats were grouped into four groups containing six animals in each group.

Group I: Non-diabetic rats with Normal diet and no dose (Normal control).

Group II: Non-diabetic rats + DMSO 10% as vehicle + 200 mg kg^{-1} bw; GTE orally.

Group III: Diabetic rats + normal diet + Single dose of STZ (55 mg kg^{-1} bw) i.p dissolved in normal saline.

Group IV: Diabetic rats + single dose of streptozotocin (55 mg kg^{-1} bw) i.p dissolved in normal saline + 200 mg kg^{-1} bw; GTE orally.

Preparation and Administration of Green Tea Extract: Green tea extract (200mgkg^{-1} bw) was fed orally during the entire tenure of the experiment using an infant feeding catheter (3 mm size) attached to a sterile syringe. The catheter was inserted into the gastric region of rats, and 200 mg kg^{-1} bw of GTE was discharged gradually into each test animal.

Determination of Body Weight, Glucose, Protein, Albumin, and Globulin: The experimental rats were weighed on 1st, 7th and 14th day using a top pan balance. The rats were anesthetized at the end of the experimental period, and samples of fasting blood were taken straight from the cardiac. For 15 min the blood samples were centrifuged at 3000 rpm, and for further biochemical analysis, the serum was separated. Serum glucose was estimated using GOD-POD method and expressed in mg/dl. Total protein was assessed using the Biuret method. Albumin and globulin were determined using dye bromocresol green and standard method [15].

Biological Assays of Total Cholesterol and Triglycerides: The serum was used for the estimation of lipid profile. Total cholesterol (TC) and triglycerides (TG) were estimated by standard procedure (direct method).

Hormonal Assays: The hormonal tests (testosterone hormone) were determined using a technique of enzyme-linked immunosorbent assay (ELISA) using the AccuBind ELISA kit (Monobind Inc. CA, USA).

Statistical Analysis: Statistical analyses of initial day, 7th day and 14th day on said parameters were carried out using Microsoft Excel 2007 and expressed in Mean ± SEM, t-test and one way ANOVA, with $p<0.05$ was considered to be statistically significant.

RESULTS:

Effect of GTE on Body Weight: Bodyweight of diabetic control was found to be decreased as compared to normal, and after administration of GTE, bodyweight improved in diabetic subjects. However, in normal subjects, when treated with GTE lowered the body weight as compared to normal.

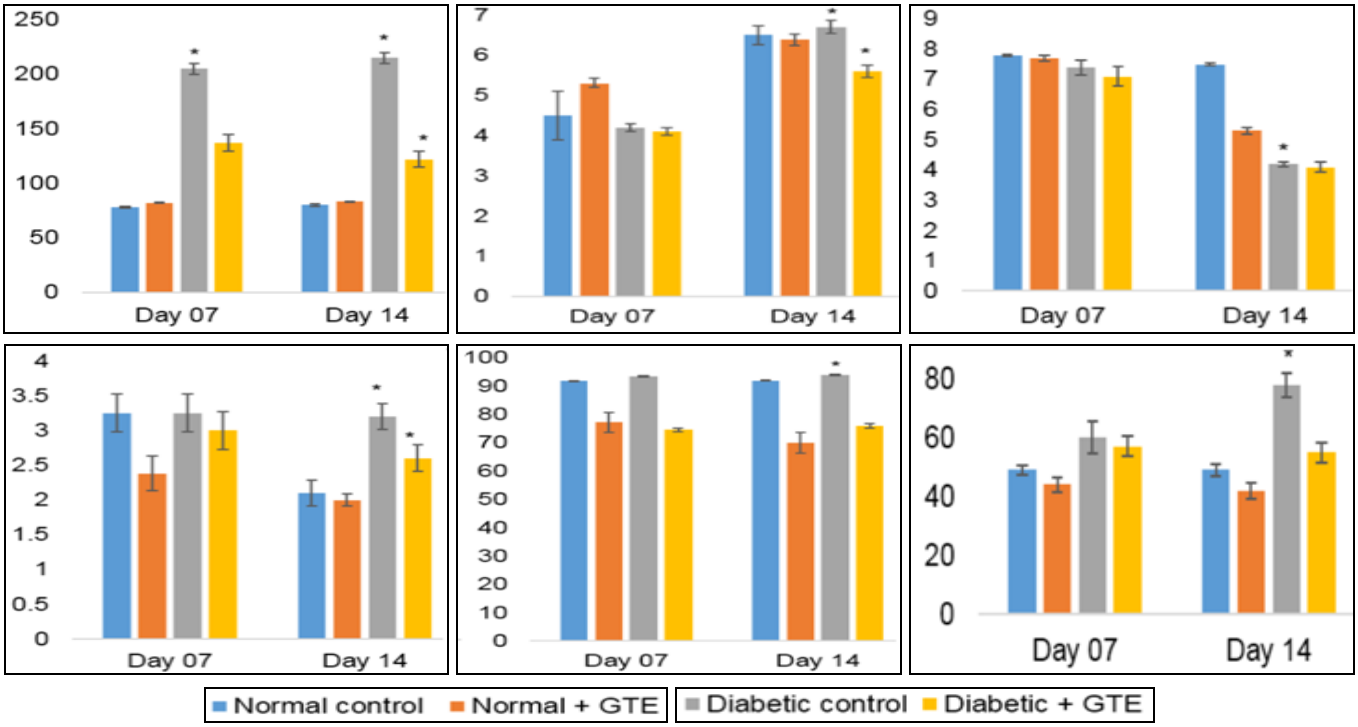

FIG. 1: GROUP I: NORMAL CONTROL, GROUP II: NORMAL + GTE, GROUP III: DIABETIC CONTROL, AND GROUP IV: DIABETIC + GTE. (A) Fasting blood glucose level (mg/dl), (B) Albumin (g/dl), (C) Protein (g/dl), (D) Globulin (g/dl), (E) Total Cholesterol(mg/dl), (F) Triglycerides (mg/dl). Values are expressed as mean ± SEM (n=6); superscript * is significantly different at $P<0.05$ respectively when compared to control. One-way analysis of variance was used followed by the *post-hoc* Tukey multiple comparison test.

TABLE 1: GROUP I: NORMAL CONTROL, GROUP II: NORMAL + GTE, GROUP III: DIABETIC CONTROL, AND GROUP IV: DIABETIC + GTE

	Body weight (gm)		Testosterone (ng/dl)
Groups	**Day 7**	**Day 14**	**Day 14**
Normal control	241±9.43	275±10.85	45±0.8
Normal + GTE	265±13.1	280±16.16	40±0.7
Diabetic control	255±8.53	265±7.59*	7.75±0.6*
Diabetic + GTE	257±5.63	226±6.77	5.68±0.3

Values are expressed as mean ± SEM (n=6); superscript * is significantly different at P<0.05 when compared to control. One-way analysis of variance was used followed by the *post-hoc* Tukey multiple comparison tests.

The results showed that body weight on 7th and 14th day in both diabetic control rats and GTE-treated diabetic rats were considerably lower than ordinary control rats, but on the 14th day of the study, the bodyweight of the diabetic rats treated with GTE increased the weight as compared to diabetic control. The decreased level of body weight in diabetic subjects was statistically significant (p<0.05).

Effect of GTE on Certain Biochemical Parameters: Results of GTE on different biochemical parameters such as blood glucose, protein, albumin and globulin values have been presented as mean ± SEM. Fasting blood glucose, albumin, triglyceride and total cholesterol were found to be increased in diabetic subjects whereas protein content and globulin showed reverse values which were further improved by GTE treatment. The decreased level of protein and increased level of glucose level in diabetic subjects were statistically significant (p<0.05).

Effect of GTE on Serum Testosterone: Effect of GTE was evaluated on the 14th day of the experiment and expressed in ng/dl. The serum testosterone of the control group ranged between 45 and 40 ng/dl, whereas treated group showed meager value in the diabetic group and diabetic treated subjects.

DISCUSSION: Diabetes mellitus is an idiopathic disease that deals with the chronic metabolic disorder involving carbohydrate, lipid, and protein metabolism alterations and by the occurrence of insulin deficiency or dysfunction. Its prevalence has been increased more rapidly day by day. DM is responsible for biochemical variation and other pathological changes that affect male fertility. The sexual dysfunctions such as spermatogenesis, retrograde ejaculation or erectile dysfunction occur and these end up with decreased sexual appetite in diabetic individuals [16].

DM results in changed pathways by multiple molecular mechanisms with dramatic consequences to male reproductive functions. DM decreased sperm quality and functioning competitively to normal. Alteration in testicular cells is concerned with glucose metabolism. Specific mechanisms related to hormonal control and glucose sensing machinery may also play a critical role in sub-fertility and fertility correlated to DM. Many other reports also identify that the DM correlates with the degradation of hormones, particularly sex hormones, reduction in motility and vitality of semen and declined semen ejaculation without the change in viscosity of sperm. Alteration in insulin can change the primary sexual glands functions and testicular functions, which cause concentrated seminal insulin than serum insulin [1, 4].

The experimental rats were divided into four groups, each group containing 06 animals. The total experimental protocol was maintained for 14 days after the induction of diabetes. A normal rat has a blood glucose level of around 100 mg/dl (ranging from 60-130 mg/dl) after 4-h of fast, which is close to humans, where 80-120 mg/dl is considered to be normal.

There was a significant reduction in body weight at day 07 and day 14 in diabetic control when compared to control rats (p<0.01). The gradual decrease in body weight in Group-IV was also noticed when compared with normal control. The results obtained in the present work clearly show the effects of GTE on the body weight in streptozotocin-induced diabetic rats. There was a decrease in body weight in Group-II, Group-III and Group-IV when compared with Group-I (p<0.05). A similar result was observed when subjects were treated with a cell suspension of *Spirulina platensis* [17]. The fasting blood glucose level of SD diabetic rats showed an increasing trend with the increasing period (07 days and 14 days).

In this study, GTE powder at a dose of 200 mg kg^{-1} bw was evaluated in normal and diabetic rats. The control animal showed 78 ± 17 - 80 ± 16 mg/100 ml of blood glucose during the experiment, which was found to be increased to 80 ± 16 mg/100ml at 14 days of Group-I. There was a decreasing trend in the blood glucose level in Group-IV when diabetic rats supplemented with GTE powder. It has been seen that treatment with GTE powder brought down FBGL from a higher value on the 14^{th} day. The results are in agreement with the other worker [18].

The total cholesterol of control rats (Group-I) ranged from 91 ± 13 mg/dl to 92 ± 23 mg/dl at day 7 to 14 days, respectively. There was a significant increase in total cholesterol at all exposure periods ($p<0.05$) in diabetic control (Group-III) when compared to control rat. The serum triglyceride of control rats showed 49 ± 4 mg/dl to 49 ± 6 mg/dl during all experimental periods. Not as much as a significant increase in triglyceride was observed at day 7 and day 14 in Group-III and Group-IV ($p<0.01$) when compared with Group-I. Diabetic rats, when treated with GTE, showed triglyceride decreased from 44 ± 5.1 mg/dl to 42 ± 4 mg/dl at day 14. Supplementation of GTE powder reduced total cholesterol and triglycerides in serum as compared to the diabetic group but insignificant at day 07.

Onakpoya *et al.*, [19] have discussed the modulation of lipid after prolonged intake of green tea. The total serum protein of control rats showed 7.8 ± 0.03 to 7.5 ± 0.04 gm/dl at 14^{th}day. The total protein in diabetic subjects was found to be decreased which was ameliorated after treatment with GTE. The reduction of total protein in serum was significant in diabetic subjects ($p<0.05$). There was an insignificant change in protein value when normal subjects are treated with GTE.

Prasanth *et al.*, 2019 [20] reported that increased consumption of green tea is related to decreased serum concentrations of total cholesterol and triglyceride. The serum testosterone of control group ranged between 45 ± 0.8 and 40 ± 0.7 ng/dl respectively, and the treated group showed drastically low down the value in diabetic group, and treatment with GTE have not so much pronounced when compared with normal.

To conclude it can be said confidently that GTE powder supplementation plays a beneficial role in maintaining blood glucose level and decreased body weight in streptozotocin-induced diabetic rats.

CONCLUSION: From this study, it can be concluded that oral treatment with GTE to diabetic rats modulates physiological and biochemical status. Results unmasked effects of GTE against DM and warrants further investigation at wider dose regimens and extended duration in other test animals.

ACKNOWLEDGEMENT: Authors are thankful to the Head and Dean, Lovely Faculty of Applied Medical Sciences, Lovely Professional University, Punjab for providing laboratory facilities, including all accessories for the present study.

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest.

REFERENCES:

1. Devi S, Singh K and Pranay PP: Prevalence, pathogenesis and diagnosis of sexual dysfunctions in diabetic women. Int J Pharm Bio Sci 2016; 7(1): 221-25.
2. Pankaj PP: Efficacy of *Spirulina platensis* in the improvement of the reproductive performance and easing teratogenicity in hyperglycemic albino mice. Indian J Pharmacol 2015; 47: 430-35.
3. Marques N and Cardoso J: 472 Sexual dysfunctions in Portuguese women with type 2 diabetes. The Journal of Sexual Medicine 2018; 15(7): S295.
4. Singh K, Devi S and Pankaj PP: Diabetes associated male reproductive dysfunctions: Prevalence, diagnosis and risk factors. Int J Drug Dev & Res 2016; 8: 7-10.
5. Ubuka T, Parhar I, Kriegsfeld LJ and Tsutsui K: The roles of GniH in reproductive function and behavior. Frontiers in Endocrinology 2018; 9: 19.
6. Rato L, Oliveira PF, Sousa M, Silva BM and Alves MG: Role of reactive oxygen species in diabetes-induced male reproductive dysfunction. In oxidants, antioxidants and impact of the oxidative status in male reproduction. Academic Press 2019: 135-147.
7. Deb A, Jayaswal RP and Pankaj PP: Extraction of flavonoids from green tea and its *in-vitro* efficacy against some selected microbes. Int J Pharm Bio Sci 2016; 7(2): (B) 744-748.
8. Solanki N, Jayaswal RP and Pankaj PP: Therapeutic efficacy of *Moringa oleifera* and *Camellia sinensis* extracts in combination against peritonitis induced rat model. IJTPR 2015; 7(3): 147-52.
9. Roychoudhury S, Halenar M, Michalcova K, Nath S, Kacaniova M and Kolesarova A: Green tea extract affects porcine ovarian cell apoptosis. Reproductive Biology 2018; 18(1): 94-98.
10. Shetta A, Kegere J and Mamdouh W: Comparative study of encapsulated peppermint and green tea essential oils in chitosan nanoparticles: Encapsulation, thermal stability, *in-vitro* release, antioxidant and antibacterial activities.

International Journal of Biological Macromolecules 2019; 126: 731-42.
11. Reygaert WC: Green tea catechins: Their use in treating and preventing infectious diseases. Biomed Res Int 2018; 2018: 9105261.
12. Saeki K, Hayakawa S and Nakano S: *In-vitro* and *in-silico* studies of the molecular interactions of epigallocatechin-3-O-gallate (EGCG) with proteins that explain the health benefits of green tea. Molecules 2018; 23(6): 1295.
13. La Vignera S, Cannarella R and Duca Y: Hypogonadism and sexual dysfunction in testicular tumor survivors. A Syst Rev Front Endocrinol (Lausanne) 2019; 10: 264.
14. Kulkarni SK: Commonly used drugs, their doses and nature of action in laboratory animals. Handbook of Experimental Pharmacology. Vallabh Prakashan; Delhi, Edition 3rd, 2005: 190-5.
15. Sood R: Clinical chemistry, blood sugar estimation. Medical laboratory technology methods of interpretations. Japyee Brothers Medical Publishers (P) Ltd.; New Delhi, Edition 5th, 1999: 433-6.
16. Priyadarshani N, Pankaj PP and Varma MC: Evaluation of estrous cycle in normal and alloxan-monohydrate induced diabetic Swiss albino mice- *Mus musculus*. Columban J Life Sci 2010; 11(1&2): 97-101.
17. Pankaj PP: Cell suspension of *Spirulina platensis* partially attenuates alloxan-induced alterations in carbohydrate and lipid metabolism in diabetic mice. Int J Pharm Sci Res 2016; 7(7): 2805-12.
18. Fu QY, Li QS and Lin XM: Antidiabetic effects of tea. Molecules 2017; 22(5): 849.
19. Onakpoya I, Spencer E, Heneghan C and Thompson M: The effect of green tea on blood pressure and lipid profile: a systematic review and meta-analysis of randomized clinical trials. Nutrition, Metabolism and Cardiovascular Diseases 2014; 24(8): 823-36.
20. Prasanth MI, Sivamaruthi BS, Chaiyasut C and Tencomnao T: A review of the role of green tea (*Camellia sinensis*) in antiphotoaging, stress resistance, neuro-protection, and autophagy. Nutrients 2019; 11(2): 474.

How to cite this article:
Singh K, Devi S and Pankaj PP: Assessment of green tea extract on testicular activity in streptozotocin-induced diabetic Sprague-Dawley rat model. Int J Pharm Sci & Res 2019; 10(11): 5063-68. doi: 10.13040/IJPSR.0975-8232.10(11).5063-68.