

Consumption of Green Tea (*Camellia sinensis*) Improves Lipid, Hepatic, and Hematological Profiles of Rats That Are Submitted to Long-Term Androgenic Stimulation

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Abstract: Indiscriminate use of anabolic steroids is associated with cardiovascular diseases, such as myocardial infarction, thrombosis, and arterial obstruction. Furthermore, high levels of androgens increase hepatic toxicity and the risk of cancer. Contrastingly, green tea prevents and controls cardiovascular and hepatic diseases, as it can improve the lipid profile and reduce inflammation and effects of oxidative stress. This study will evaluate benefits of green tea consumption, to attenuate systemic damage caused by supraphysiological doses of testosterone, by analyzing the lipid, biochemical, and hematological profiles of 28 42-day-old male Wistar rats. Silicone pellets containing testosterone in proportion were surgically implanted and replaced in these rats every four weeks, and they received casein-based control feed and water or green tea for hydration. After 20 weeks, all the male rats were anesthetized and their blood samples collected for the analysis of their biochemical and hematological profiles. Although the high hormone concentration had a negative influence on the lipid profile of these animals, the groups that consumed green tea exhibited a reduction in serum triglycerides (62%), Low Density Lipoprotein (76%), and Very Low Density Lipoprotein (45%). Tea consumption also led to a significant reduction in total cholesterol (32% in the green tea control group and 45% in the green tea-induced group), without changing the High Density Lipoprotein fraction. Only the green tea-induced group manifested a reduction in the total concentration of serum proteins. A fall in serum albumin was observed in the green tea-induced groups (2.3 g/dL) compared to control groups (2.9 g/dL). The induced group presented elevation in hematocrit, erythrocytosis, and leukocytosis in contrast to the green tea-induced group. The green tea control group maintained erythrocytosis, but without any other potentially harmful effect. A 30% increase in lymphocyte population in the induced group was observed. There was no difference in the platelet count of these rodents. Hepatic enzymes were also shown to have increased in the induced group, indicating hepatic injury in this group due to exposure to testosterone. This effect was reversed in the tea groups. From this, its possible to reach the conclusion that consumption of green tea shields the lipid profile, proteins, liver enzymes and hematological profile, thus reducing risk factors related to the supraphysiological doses of testosterone.

Keywords: Testosterone Green, Tea, Cardiovascular Diseases, Lipid Profile, Hematological Profile, Hepatic Cancer

1. Introduction

In the last three decades, there is a continuous growth in the use of anabolic steroids both by high performance athletes and adolescents as well as young non-athletes [1–6]. Several youngsters engage with anabolic steroids before the age of ten [5]. Steroid abuse has effects on the physiology of cardiac and vascular tissues [7–9]. Several authors demonstrated the positive association between testosterone use, as an anabolic agent, and the occurrence of cardiovascular disease (CVD) [10, 11]. In many cases, random use of anabolic steroids may lead to increased blood pressure, myocardial infarction, and even death of individuals without previous diseases, [3, 6, 12, 13] besides other dysfunctions that degrade the user's quality of life.

On the other hand, the consumption of nutraceutical foods can assist in the better evolution of weight loss, body fat reduction, and overall improvement of organic functions. Green tea (*Camellia sinensis*) [14] comprises components that act on the body's systems and can reduce and/or retard the severity or evolution of some diseases. In its composition, the green tea contains antioxidants and anti-inflammatory elements [15] that are capable of altering lipids and cholesterol metabolism, decreasing risks of CVD [16], and modulating the metabolism of testosterone [17, 18]. Appropriate nutrition, which includes this type of food and is associated with physical exercise, indicates prophylaxis or even an additional part of the therapy of some diseases, especially CVD.

Thus, based on the gained knowledge on the actions of testosterone and benefits of green tea as a nutraceutical, this study aims to evaluate the biochemical and hematological effects of green tea consumption in animals that are submitted to prolonged androgenic stimulation with supraphysiological doses of testosterone.

2. Materials and Experiments

2.1. Animals

The use of animals was approved by the Animal Use Ethics Committee of the Federal Fluminense University (RJ, Brazil) under the protocol, CEUA-UFF-765/2016. Materials from 28 adult male Wistar rats aged 42–50 days were used. The rodents in this study were subdivided into four groups of seven rodents each and arranged as follows: control group (CG) — animals hydrated with water; green tea group (GTG) — animals hydrated with green tea; induced group (IG) — animals that received testosterone and were hydrated with water; and induced green tea group (IGTG) — animals that received testosterone and were hydrated with green tea. The rodents were housed in individual plastic cages at a 12:12-h light/dark cycle and constant temperature of 22±1°C, with free access to water or green tea, and received casein-based feed, *ad libitum*. Body weight and feed and water/green tea intakes were monitored weekly.

2.2. Induction

Induction with testosterone was performed by using silicone pellets (Dow Corning, cat. no. 508-009 Silastic™ Tube, 1.98 mm I.D.×3.18 mm O.D., 5 cm long) filled with propionate testosterone (1 mg) and sealed with a surgical adhesive [19]. The rodents were anesthetized intraperitoneally with xylazine (10 mg/kg) and ketamine (75 mg/kg), and the pellets were implanted in their dorsal scapular region with an incision of approximately 10 mm. For a skin synthesis, a cyanoacrylate-based surgical adhesive was used. The pellets were replaced every four weeks for 20 weeks.

2.3. Control Feed

The control casein-based feed was isocaloric and had a vitamin and mineral mix added to it in accordance with the recommendations of the American Institute of Nutrition (AIN-93M) [20]. The ingredients in the control feed (Table 1) were weighed and homogenized with a Hobart® industrial mixer (São Paulo, SP, Brazil) — with boiling water for the gelatinization of the starch. The obtained dough was transformed into the pellets and dried in a ventilated incubator (Fabbe-Primar® n°171, São Paulo, SP, Brazil) at 60°C for 24h and stored under refrigeration until use, after identification.

Table 1. Composition of every 100g of feed used in the study.

Nutrients	g/100g
Casein ^a	14,00
Starch ^b	58,95
Refined sugar ^c	10,00
Mineral mix ^a	3,50
Vitamin mix ^a	1,00
Soybean Oil ^d	7,00
Cellulose ^e	5,00
Choline bitartrate ^a	0,25
Cystine ^a	0,30
Tert-Butylhydroquinone ^a	0,0014

The ingredients used in the preparation of the diet were supplied by: [a] M. Cassab Industry and Commerce Limited (São Paulo, SP, Brazil). [b] Maizena Unilever Bestfoods Brazil Limited (Mogi Guaçu, SP, Brazil); [c] União (Rio de Janeiro, RJ, Brazil); [d] Liza Cargill Agriculture Limited (Mairinque SP, Brazil); [e] Microcel Blanver Limited (Cotia, SP, Brazil).

2.4. Green tea Preparation

The tea was prepared by infusing the leaves of *Camellia sinensis* (Yamamotoyama-Midori n°262, São Miguel Arcanjo, SP, Brazil) at a concentration of 2%, calculated by weight/volume [21]. For this, 20 g of leaves were used for 1000 ml of water. In this protocol, the leaves are infused for two minutes and, then, the tea is filtered and cooled immediately. The animals in the green tea groups had their hydration exclusively through tea, and the supply was 200 ml/day.

2.5. Biochemical and Hematological Profile

At the end of the experimental period at the bioterium, the animals were euthanized. The rodents were anesthetized with 75 mg/kg of ketamine plus 10 mg/kg de xilazine, and the calculated dose was intraperitoneally administered. After the realization of the anesthetic condition through the absence of pedal reflex, the rats were then submitted to bleeding by intracardiac puncture, from which 10 ml of blood was obtained. After bleeding, an additional dose of the anesthesia was administered, which led to the death of the rodent. Blood samples containing anticoagulants were immediately used for hematological analysis. Blood samples collected without Ethylene diamine tetra-acetic acid (EDTA) were held for about two hours at room temperature for clot retraction. Thereafter, they were centrifuged at 958.5 G for five minutes to obtain the serum and stored at 20°C. Biochemical analyzes (of albumin, total proteins, cholesterol, triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)) were performed using LabTest colorimetric kits (LabMax, Belo Horizonte, Brazil). The serum testosterone was measured by radioimmunoassay (RIA) using a commercial, solid-state Beckman Coulter kit (Immunotech®). The tests were performed in the laboratory of the Brazilian Institute of Diagnosis and Veterinary Specialties (PROVET / São Paulo, Brazil), using the Perkin Elmer (RIA) WIZARD2 equipment. All parameters of quality of the essay were performed in accordance with instructions from the international scientific community.

2.6. Statistical Analysis

The data were presented under the average and standard

deviation form. To test the normal distribution of values, the Kolmogorov-Smirnov test was deployed and, for data analysis, the ANOVA univariate test was deployed, which is associated to the Tukey-Kramer multiple comparison test. The significance in all tests was established to the level of $p < 0.05$. The statistical analyses were performed by the Graph Pad Prism version 5.0, 2007 (San Diego, C.A., U.S.A.) program.

3. Results

3.1. Biochemical Profile

The serum testosterone concentration increased by a 110% in the IG when compared to the values observed in CG. The GTG and IGTG presented seric testosterone values close to CG values. A reduction of the serum total protein, accompanied by a significant reduction of serum albumin, could be visualized in the groups hydrated by green tea. The serum concentration of liver transaminases, ALT and AST, was found to be higher in animals in the induced group — an effect that was reversed in animals that consumed green tea.

There was a significant increase in the serum triglyceride value (TG) of the IG group (153±24.01) when compared to the CG (106.8±23.6). The concentration of serum TG decreased by about 50% with the administration of green tea. When compared with the IG, serum cholesterol decreased about 40% in GTG and IGTG. The serum concentration of HDL cholesterol remained stable in the groups that received green tea. The IG manifested an increase in HDL and other cholesterol fractions and total cholesterol increased proportionally. Seric LDL in IG was found to be five times higher than that in GTG, IGTG, and CG. The VLDL values in the green tea groups were significantly lower (about 50%) when compared to the IG and CG (Table 2).

Table 2. Biochemical profile of the experimental groups.

Parameters	Groups				P value
	CG	IG	GTG	IGTG	
Testosterone (ng/dL)	314,35±74,19	661,86±190*	332,66±157,54	270,14±155,67	0,0031
Total proteins (g/dL)	6,33±0,22	6,88±0,31	6,55±0,73	5,26±0,88*	0,0011
Albumin (g/dL)	2,93±0,44	2,97±0,23	2,31±0,34*	2,31±0,31*	0,0006
ALT (UI/L)	15,19±5	18,33±4*	10,25±2	10,23±2	0,0001
AST (UI/L)	47,3±15	53,33±5	45,57±15	33,2±8	0,1825
Triglycerides (mg/dL)	106,8±23,6	153±24,0*	59,85±24,0*	57,16±23,0*	<0.0001
Cholesterol (mg/dL)	55,51±15,9	60,84±13,9	37,42±11,9*	33,5±5,8*	<0.0001
HDL (mg/dL)	22,35±3,83	26,72±5,42	17,14±4,70*	16,83±4,99*	<0.0001
LDL (mg/dL)	4,47±3,57	21,93±8,84*	8,31±7,36	5,23±1,11	<0.0001
VLDL (mg/dL)	23,28±5,78	21,14±5,87	11,97±4,81*	11,43±4,60*	<0.0001

*significant difference

3.2. Hematologic Profile

A significant increase in the number of red blood cells (RBC) and hematocrit value in IG was observed. The GTG had higher number of red blood cells and hematocrit than CG and IGTG. Hemoglobin values remained close to control, except those in the IGTG. In qualitative analysis, all groups

showed discrete anisocytosis. The red cell distribution width (RDW) values were higher in the IG, GTG, and IGTG than in the CG. The leukocyte count was two times higher in the IG compared to the other groups. The animals in IG presented predominance (average 80%) of lymphocytes in the leukocyte population. There was no significant differences in the platelet count of the animals (Table 3).

Table 3. Hematologic profile observed in the experimental groups.

Parameters	Groups				p Value
	CG	IG	GTG	IGTG	
Red Blood Cells (*10 ¹² /L)	7,62±0,31	8,84±0,45*	8,46±0,76*	7,96±0,44	0,0008
Hematocrit (%)	42,1±1,9	49,8±1,2*	44,0±3,0*	41,3±2,1	0,0001
Hemoglobin (g/dL)	14,7±0,6	14,38±0,5	14,0±0,9	13,3±0,6*	0,0001
RDW (%)	13,65±0,7	15,66±0,4*	15,65±0,4*	15,78±0,6*	0,0001
Leukocyte (*10 ⁹ /L)	3,62±1,33	6,37±1,0*	3,71±1,64	3,96±0,90	0,0001
Lymphocytes (%)	58,65±10,9	80,17±4,0*	48,14±6,5	47,33±5,2	0,0001
Monocytes (%)	3,7±2,5	3,0±2,4	0,71±0,75	0,83±0,75	0,0001
Segmented (%)	35,32±9,4	16,66±5,6*	50,57±5,7	51,66±5,54	0,0001
Platelets (*10 ⁹ /L)	689,37±27	609,5±89	530,57±62	563,5±13	0,3272

*Significant difference.

4. Discussion

Serum testosterone concentration in the control group was similar to the reference values for Wistar rats [22, 23]. Increased testosterone concentration in the IG shows that the induction was satisfactory. The IGTG presented low serum testosterone values, but close to control group. These data confirm the literature, demonstrating the anti-androgenic effects of the consumption of polyphenols extracted from green tea, either by the modulation of the 5-alpha-reductase enzyme or other pathways [18, 24, 25].

An increased concentration of serum triglycerides in the presence of androgenic supraphysiological stimulus, with the use of anabolic steroids, is expected. This effect can be visualized in humans despite the practice of physical exercises [26]. The administration of steroids, even for a short term, produces unfavorable effects not only on lipases but also on apolipoproteins related to HDL [27], besides raising the low-density fractions of cholesterol [26, 28, 29]. Together, these factors significantly increase the risk of cardiovascular disease. But if on the one hand testosterone was able to increase triglycerides, cholesterol, LDL, and VLDL, this study showed that in contrast, green tea consumption improved the lipid profile, even with androgenic stimulus. It is already known that polyphenols and other antioxidants of tea have a great capacity to reduce not only the cholesterol and fraction concentration but also their oxidation [30, 31]. This became evident when the triglyceride values reduced in green tea groups by about 50%, in relation to the control, apart from the decreased concentration of total cholesterol, LDL, and VLDL. HDL fraction in tea groups, however, remained stable. In addition to the beneficial effect on the lipid profile, green tea consumption reduces the accumulation of lipids in the liver and improves glucose tolerance, thus further decreasing the risk of developing metabolic diseases and CVD [32–35].

The serum total protein and albumin in CG were within the reference values [22]. When compared with CG, the serum protein and albumin concentrations had slightly increased in IG whereas, in the tea groups, seric proteins and albumin had decreased. One of the hypotheses for increased protein in IG would be the pro-inflammatory potential of testosterone over the body. It has already been shown that testosterone administration

promotes inflammatory response as well as release of cytokines, a fact that would be an explanation for the observed results [36–38]. In contrast, it has already been observed that consumption of green tea led to the improvement of various inflammatory parameters, including total proteins [39]. Protein reduction in the GCCV and GICV groups could be indicative of the anti-inflammatory potential of green tea, which would reduce the number of inflammatory mediators in the body, reducing the total protein in both control and induced animals.

In addition to the inflammatory potential, high concentrations of testosterone may be harmful to liver tissue [40, 41]. Increased hepatic enzyme concentration in induced animals indicates that testosterone causes tissue damage, which is potentially harmful. It is a fact reported in the literature that green tea is beneficial for liver health [16, 42]. The administration of green tea was also beneficial in this study, as it significantly reduced indicators of hepatic damage in the animals of the GTG and IGTG. The use of anabolic agents increases the risk of developing hepatic neoplasms, with a serious prognosis for the user [43, 44]. The consumption of green tea, as observed by its effects, can protect the liver and even be an ally in the therapy against hepatic neoplasms [45].

Regarding the hematological profile, it is expected that testosterone stimulus will increase erythropoiesis, hemoglobin, and hematocrit [46], as one of the expected benefits of steroid use is improved oxygenation and faster recovery of the tissue [47]. The androgenic stimulus has already been tested as an alternative treatment for anemias that occur as a consequence of severe diseases [48] because in addition to erythropoiesis, testosterone acts on iron metabolism [47]. In this study, the values of red blood cell count, hematocrit, and hemoglobin of the CG were within the reference range for male rats [22] and, in the IG, a significant increase in both red blood cell count and the hematocrit value was observed. The GTG group had positive hematological parameters, which indicates that the ingestion of green tea could improve the hematological profile, but without causing damage. A slight reduction of hemoglobin in the IGTG was observed. Similar results were found by Marouani [49], who stated that this apparently negative result of hematological parameters in individuals who consumed green tea as infusion is not due to the catechins contained in it, but due to

the absorption of other components released during cooking, which would alter the absorption of iron.

Leukocytosis observed in the IG, not visualized in the other groups, may be an effect of acute inflammatory processes that occur as adverse effects of the use of anabolic agents. The literature shows that the use of anabolics is related to acute inflammation in the skin, heart [50], blood vessels [51], kidney [52], liver [53], and other organs and that leukocyte recruitment increases with use of supraphysiological doses of steroid hormones in rats [38]. In addition, it has been shown that in normal rats receiving androgenic stimulation, not only leukocytosis but there is also a decrease in cellularity in the thymus and bone marrow [54], which corroborates the hypothesis that the supraphysiological hormone dose not only increases lymphocyte production but also stimulates its differentiation and recruitment. Fijak [55] reaffirm this result, showing that hormone replacement with high doses of testosterone in vivo generates leukocytosis, with expansion of regulatory T lymphocytes and B lymphocyte—which could explain the increase in the lymphocyte population of the hyper stimulated group—besides increasing the chemotaxis of these cells, mediated by MCP-1. The IGTG had a lower lymphocyte count, when compared with IG, close to the control group. This result is probably due to the antioxidant potential of the polyphenols contained in green tea which, in a pro-inflammatory context such as obesity, has already been shown to reduce the production of cytokines and decrease the chemotaxis of inflammatory cells in rats [56, 57].

5. Conclusion

The consumption of green tea attenuates the systemic damages caused by supraphysiological concentrations of androgens, improving liver parameters, lipid metabolism, and hematological parameters, thus indicating the prevention and aid in the treatment of diseases caused by prolonged androgenic stimulation; and their consumption by healthy individuals is also safe and advantageous.

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