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The role of green tea extract and powder in mitigating metabolic syndromes with special reference to hyperglycemia and hypercholesterolemia

S. Yousaf,[a] M. S. Butt,[a] H. A. R. Suleria*[b] and M. J. Iqbal[a]

Natural products are currently gaining popularity to combat various physiological threats. Scientific evidence has been provided that dietary phytochemicals may play important roles as chemo-preventive or chemotherapeutic agents in the prevention of many diseases. Green tea has many biologically active moieties, like flavanols and polyphenols. Catechins are flavanols that constitute the majority of soluble solids of green tea; its major components are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). Among these, EGCG is the predominant component, contributing more than 50% of polyphenols. It has many health related characteristics, like hypoglycemic, hypocholesterolemic, anticancer, antiviral and antihypertensive activities. Ethanolic extracts of green tea was subjected to *in vivo* modeling. An efficacy trial was carried out on normal, hyperglycemic and hypercholesterolemic rats for 8 weeks. Control, functional and nutraceutical diets were used for each study. Drink and feed intake and body weight increased during the study period. Serum analysis showed that maximum reduction of cholesterol level was noted in hypercholesterolemic rats, up to 15.45%, due to the nutraceutical diet. It was a 21.51% reduction in the case of LDL and 12.92% for triglycerides. The serum glucose level was most reduced in hyperglycemic rats, up to 13.39% as a result of the nutraceutical diet. The functional diet resulted in a bit less reduction in the respective traits compared to the nutraceutical diet. Hematological analysis revealed that administration of green tea did not adversely affect the red blood cell, white blood cell and platelet count of the rats. The current research work enables us to conclude that green tea is effective against hypercholesterolemia and hyperglycemia.

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Background

The modern sedentary lifestyle includes a diet rich in fat and with a lesser proportion of dietary fiber, bioactive ingredients and micronutrients. These eating habits are the reason for an increase in diet-related ailments, mainly hypertension, obesity, diabetes, cardiovascular diseases, hyperglycemia, hypercholesterolemia, osteoporosis and cancer. Many scientific investigations have emphasized the association of a diet rich in fruit and vegetables with good health. Intake of certain active ingredients, like flavonoids, carotenoids and glucosinolates *etc.* also possesses a direct link with reduced risks of hypercholesterolemia, hypertension, cardiovascular disease and many other chronic illnesses.[1,2]

Tea is a beverage prepared from the leaves and buds of the plant *Camellia sinensis* and is the second most consumed beverage worldwide after water; well ahead of coffee, beer and carbonated soft drinks.[3,4] It is variably consumed across the globe as green, black, or oolong tea. However, amongst all these, green tea intake is reported to have a remarkable significance on human health.[5] Despite the economic and social interest, tea is consumed as a part of the daily routine of many humans, as an everyday drink and also as a therapeutic aid against many ailments.

Green tea is manufactured in such a manner that avoids the oxidation of all green leaf phenolic compounds. However, oolong tea is a semi-oxidized product, whereas oxidation is encouraged during black tea production so that most of these substances are oxidized. As a standard, each year 2.5 million metric tons of dried tea is manufactured across the world. Out of this, green tea comprises only 20% and is mainly consumed in Asian countries, like Japan, China, Korea, India and Pakistan.[6] Currently, 90% of green tea comes from China and

[a]*National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan*

[b]*School of Agriculture and Food Sciences, The University of Queensland, Hartley Teakle Building, Room C505, Brisbane, QLD 4072, Australia. Tel: +61 7 336 56335; +61 470 439 670. E-mail: ibrahim_ansar40@yahoo.com*

Chinese people strongly believe that green tea consumption is better than medication.[7]

Catechins constitute a greater part of green tea soluble solids; the major components include epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). Among these, EGCG is the predominant element, contributing more than 50% of the total polyphenols.[8] Green tea also contains carotenoids, tocopherols, ascorbic acid and minerals such as Cr, Mn, Se or Zn.[9] Other components present in tea include protein, carbohydrates, lipids, sterols, vitamins, xanthic bases (caffeine and theophylline), volatiles compounds, minerals and trace elements.[5]

Additionally, catechins exhibit antimutagenic, hypoglycemic, antiinflammatory, anticarcinogenic, antibacterial and antiviral properties.[5,7,10,11] The antioxidant properties of polyphenols are the key contributing factors attributed to tea and in Japan catechin-rich beverages are available under the label of "Food for Specified Health Use". Approximately 34% of the total polyphenol utilization from beverages in Japan comes from green tea.[12–14] However, tea polysaccharide conjugates (TPC), a potentially active fraction, is much more popular internationally for its antidiabetic and immunomodulatory activities, especially in hyperglycemic mice or rats.[2] Extract of green tea is known to improve insulin resistance in high-fat-fed and high-fructose-fed rodents and increase insulin sensitivity in male Sprague Dawley rats.[15,16] Studies also revealed that the regular consumption of tea polyphenols may also contribute to the prevention of type-2 diabetes.[17]

Recent research illustrates the fact that tea contributes a positive effect in consumer health such as reduced cholesterol and glucose levels, control of hypertension *etc.* whilst EGCG in particular has preventive effects against various chronic diseases.[12] Also, green tea may lower the risk of cardiovascular disease and cancer, besides other beneficial consequences for health.[5] The therapeutic activities of green tea are associated with its constituents, like tea polysaccharide conjugates (TPC), tea polyphenols (catechins), tea pigments, caffeine and theanine.

Material and method

Preparation of raw material

Green tea leaves of the Qi-Men variety were obtained from the National Tea Research Institute (NTRI), Shinkiari, Mansehra. Reagents (analytical and HPLC grade) and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan). Male Sprague Dawley rats used in the efficacy trials were acquired from the National Institute of Health (NIH) Islamabad. Diagnostic Kits used were from Sigma-Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia). Green tea dried leaves were cleaned in order to remove dust, stones and straw. Green tea leaves were ground (Renker, Model: GMO 1 grinder) and analyzed for their quality attributes as approximate composition, mineral composition, polyphenols and antioxidant capacity.

Extract preparation of green tea

Ethanolic extract of green tea was prepared using (50% v/v) water and ethanol at 50 °C for a time interval of 45 min, according to the methods described by Rusak *et al.*[18] and Aspé & Fernández[19] with some modification. Later on, the extract was filtered and subjected to rotary evaporation (Eyela, Japan).

Efficacy study

For animal trial modeling sixty male Sprague Dawley rats were procured from National Institute of Health (NIH), Islamabad and housed in the Animal Room of the National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan. During whole experimental period, temperature (23 ± 2 °C) and relative humidity (55 ± 5%) of the Animal Room was controlled with 12 hours light–dark period alternatively. At the initiation of study, some rats were sacrificed under ether anesthesia to get baseline values for each study. Three types of studies were conducted separately using normal diet, high cholesterol diet and high sucrose diet (Fig. 1) in order to determine the therapeutic effect of green tea powder (10%) and extract (5%) on selected parameters of collected sera of rats, including lipid profile, glucose and insulin levels. Each study was comprised of fifteen rats, in which each group consisted of five rats. The composition of diet given to rats is described in Table 1.

It is assured that all the experimental trials were performed in compliance with the relevant laws and institutional guidelines of the National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan. Furthermore, all the experimental modeling includes safety and dietary plans were reviewed and approved by the institutional committee(s).

Feed and water intake

The average feed intake of each group was measured on a daily basis by eliminating spilt diet from the total diet given during the whole study period.[20] The water intake for each group was also recorded on a daily basis.

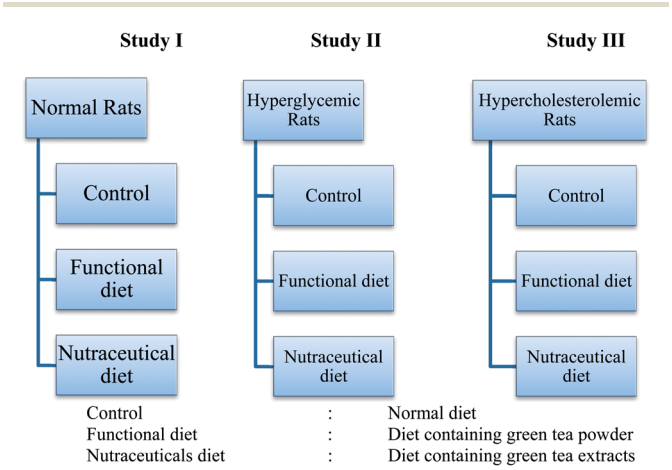

Fig. 1 The efficacy study plan.

Table 1 Composition of experimental diet

Ingredients (%)	Normal diet	High sucrose diet	High cholesterol diet
Corn oil	10	10	10
Corn starch	66	26	65
Casein	10	10	10
Cellulose	10	10	10
Salt mixture	3	3	3
Vitamins	1	1	1
Cholesterol	—	—	1
Sucrose	—	40	—

Body weight gain

Increase in body weight of rats from all experimental groups was measured weekly throughout the study period to analyze the effect of functional and nutraceutical diets on body weight.

Serum lipid profile

Serum lipid profiles of rats, including cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL) and triglycerides were measured by following their respective protocols. Details for each parameter are given below.

a. Cholesterol. Serum cholesterol level of rats was measured using the CHOD-PAP method following the protocol of Kim *et al.*[21]

b. High & low density lipoprotein. High density lipoprotein (HDL) and low density lipoproteins (LDL) in serum samples were calculated by the method mentioned by Alshatwi *et al.*[22]

c. Triglycerides. Triglycerides in serum samples were estimated by the liquid triglycerides (GPO-PAP) method as illustrated by Kuo *et al.*[23]

d. Serum glucose and insulin levels. For each study, the collected sera were evaluated for glucose concentration by the GOD-PAP method as described by Kim *et al.*,[21] whereas insulin level was assessed following the method of Ahn *et al.*[24]

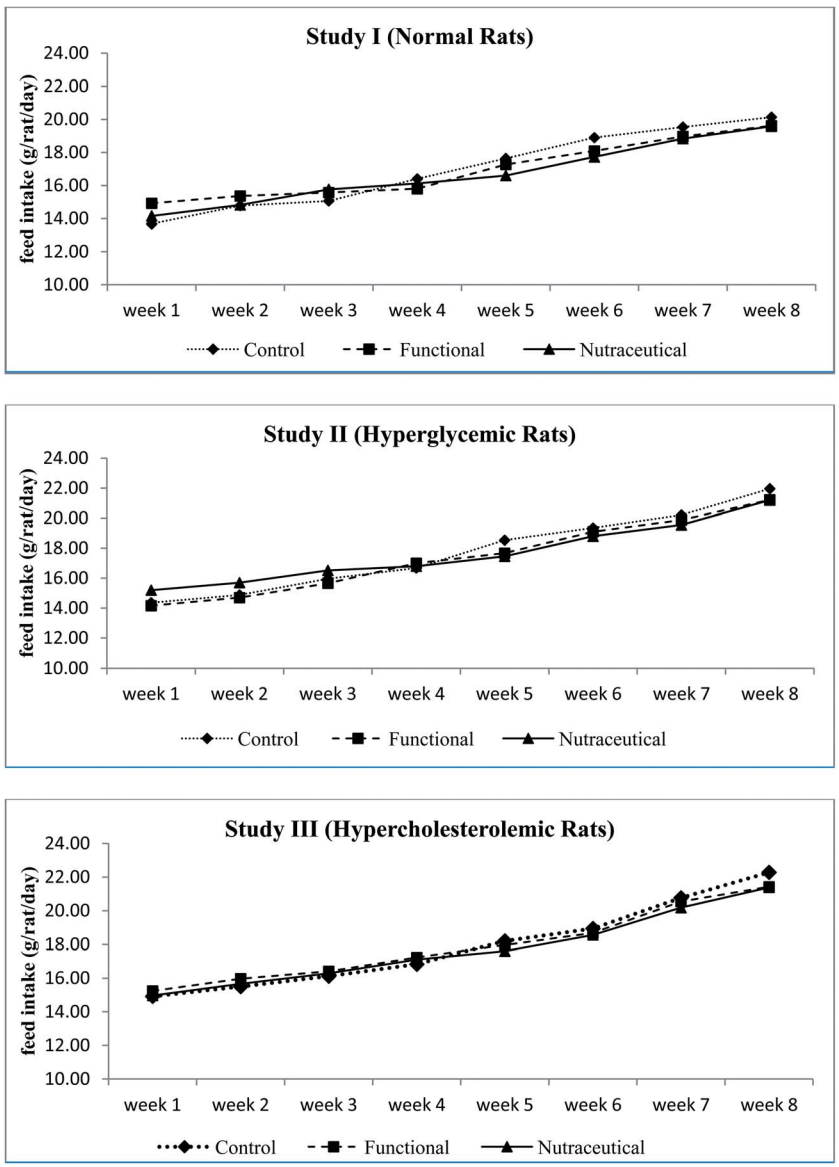

Fig. 2 Feed intake in different studies (g per rat per day).

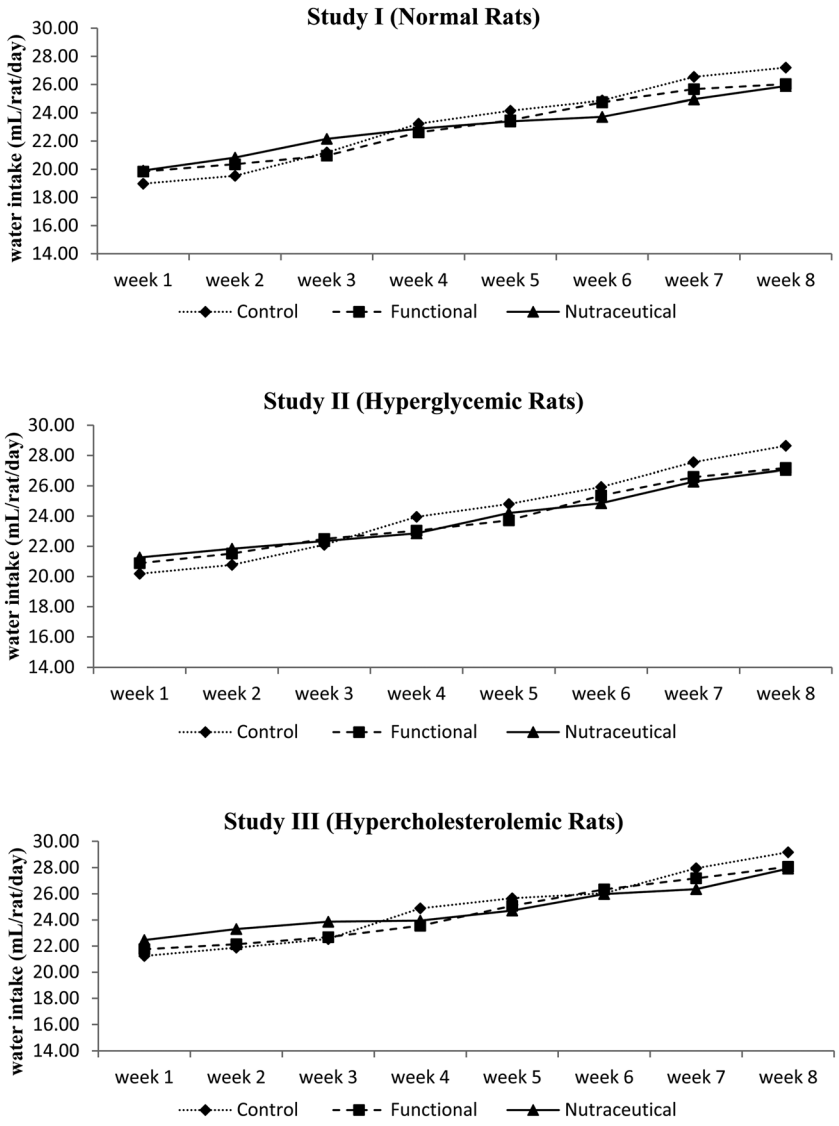

Fig. 3 Water intake in different studies (mL per rat per day).

Hematological analysis

Red blood cell (RBC) and white blood cell (WBC) indices were determined by the method of Al Haj *et al.*[25] Platelet count estimation was carried out following the method of Kamatani *et al.*[26]

Statistical analysis

The data for each parameter was subjected to statistical analysis to determine the level of significance.[27] Analysis of variance was calculated using factorial design whilst means were interpreted by Latin square design.

Results and discussion

Efficacy studies

Efficacy studies were carried out *in vivo* on Sprague Dawley male rats in order to estimate the functional and nutraceutical significance of green tea powder and its prepared extract against hyperglycemia and hypercholesterolemia. The reasons for using rodent experimental modeling instead of human subjects include easy handling, apt supervision, and feasibility of controlled environmental conditions and safety concerns regarding the active ingredient being used.

Feed and drink intake

Mean square values for feed intake indicated significant differences depending on trial group and experimental time span; means regarding feed intake are presented in Fig. 2. In study I, feed intake of control group rats was found to increase successively throughout the trial interval whilst a lesser increase was recorded in rats consuming a nutraceutical diet. At the end of the study, the control group persisted with the highest feed intake (20.14 ± 0.61 g per rat per day) whereas the nutraceutical group showed the lowest average feed intake values (19.58 ± 0.48 g per rat per day). Means for diet intake, however, increased consistently during the two months in all the groups. At the end of the 8^{th} week, the mean feed intake values for study II revealed

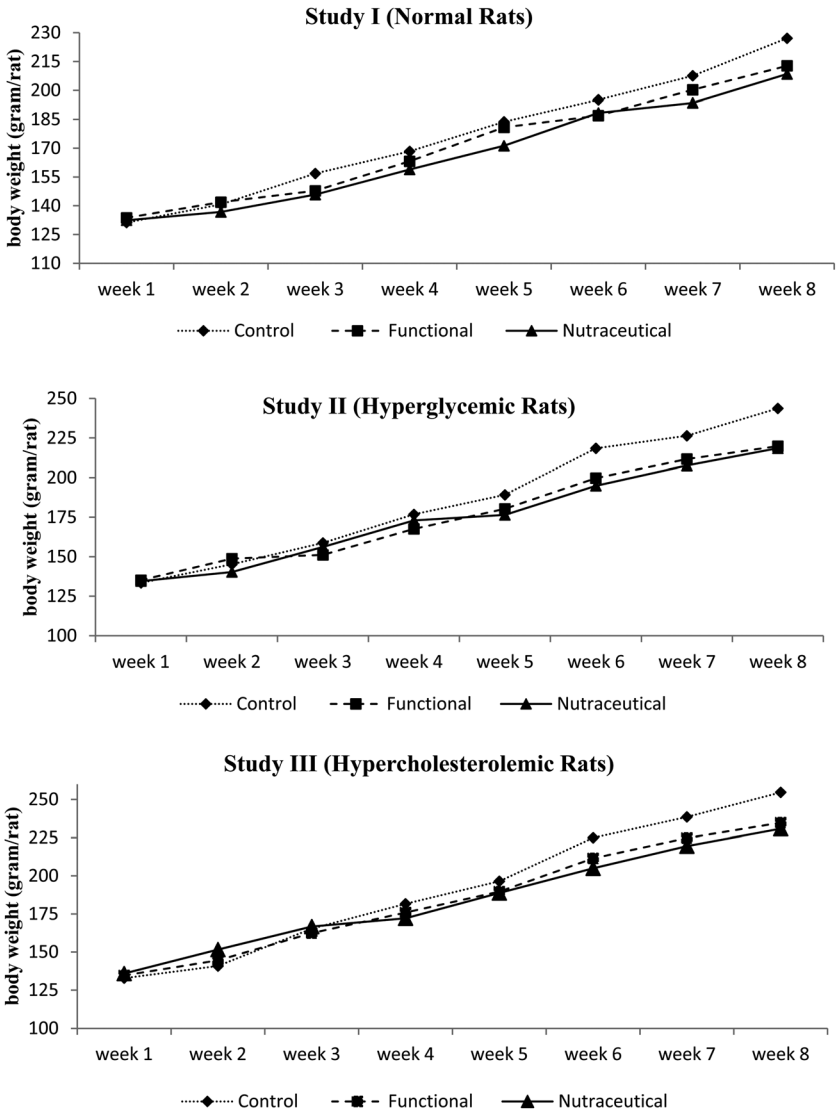

Fig. 4 Body weight in different studies (g per rat).

Table 2 Means for cholesterol (mg dL^{-1}) of rats in different studies[a]

Studies	Diet	Study intervals (days) 0	28	56	Means
Study 1	Control	81 ± 2	82 ± 2	82 ± 2	82 ± 1
	Functional	80 ± 2	79 ± 1	77 ± 1	79 ± 1
	Nutraceutical	81 ± 1	78 ± 2	77 ± 1	79 ± 1
	Means	81 ± 2	80 ± 2	79 ± 2	
Study II	Control	110 ± 3	116 ± 2	121 ± 3	116 ± 3
	Functional	108 ± 2	104 ± 2	98 ± 2	103 ± 2
	Nutraceutical	111 ± 2	101 ± 1	98 ± 2	103 ± 2
	Means	110 ± 2	107 ± 2	106 ± 1	
Study III	Control	129 ± 3	137 ± 3	146 ± 3	137 ± 2
	Functional	130 ± 3	121 ± 3	114 ± 2	122 ± 1
	Nutraceutical	131 ± 2	119 ± 2	110 ± 3	120 ± 2
	Means	130 ± 3	126 ± 2	123 ± 2	

[a] Study I: normal rats. Study II: hyperglycemic rats. Study III: hypercholesterolemic rats.

Table 3 Means for HDL (mg dL^{-1}) of rats in different studies[a]

Studies	Diet	Study intervals (days) 0	28	56	Means
Study I	Control	34 ± 0.8	34 ± 0.7	34 ± 1	34 ± 0.6
	Functional	34 ± 0.6	34 ± 0.4	34 ± 0.9	34 ± 0.9
	Nutraceutical	35 ± 0.9	35 ± 0.6	35 ± 0.5	35 ± 0.8
	Means	34 ± 0.8	34 ± 0.3	35 ± 0.6	
Study II	Control	39 ± 1	39 ± 0.8	38 ± 0.6	39 ± 1
	Functional	39 ± 0.9	39 ± 1	39 ± 0.9	39 ± 0.8
	Nutraceutical	39 ± 1	40 ± 0.9	40 ± 1	40 ± 0.9
	Means	39 ± 0.9	39 ± 1	39 ± 1	
Study III	Control	46 ± 1	46 ± 1	45 ± 0.9	46 ± 1
	Functional	47 ± 0.9	48 ± 1	48 ± 1	48 ± 1
	Nutraceutical	48 ± 1	49 ± 1	49 ± 0.8	49 ± 1
	Means	47 ± 1	47 ± 1	48 ± 0.6	

[a] Study I: Normal rats. Study II: Hyperglycemic rats. Study III: Hypercholesterolemic rats.

Table 4 Means for LDL (mg dL^{-1}) of rats in different studies[a]

Studies	Diet	Study intervals (days) 0	28	56	Means
Study I	Control	33 ± 0.8	33 ± 0.9	33 ± 0.5	33 ± 0.4
	Functional	32 ± 0.6	31 ± 0.7	30 ± 0.2	31 ± 0.6
	Nutraceutical	32 ± 0.7	30 ± 0.5	29 ± 0.6	30 ± 0.7
	Means	32 ± 0.6	31 ± 0.9	31 ± 0.7	
Study II	Control	55 ± 0.9	59 ± 1	62 ± 1	59 ± 1
	Functional	56 ± 1	52 ± 1	48 ± 0.8	52 ± 1
	Nutraceutical	57 ± 1	50 ± 1	47 ± 0.8	51 ± 0.8
	Means	56 ± 0.9	54 ± 1	52 ± 1	
Study III	Control	63 ± 1	68 ± 1	72 ± 1	68 ± 1
	Functional	65 ± 1	58 ± 0.9	54 ± 1	59 ± 1
	Nutraceutical	63 ± 1	55 ± 1	50 ± 1	56 ± 1
	Means	64 ± 1	60 ± 1	59 ± 1	

[a] Study I: normal rats. Study II: hyperglycemic rats. Study III: hypercholesterolemic rats.

Table 5 Means for triglycerides (mg dL^{-1}) of rats in different studies[a]

Studies	Diet	Study intervals (days) 0	28	56	Means
Study I	Control	71 ± 2	71 ± 2	72 ± 1	71 ± 2
	Functional	71 ± 2	70 ± 2	68 ± 1	70 ± 2
	Nutraceutical	71 ± 2	69 ± 1	68 ± 2	69 ± 1
	Means	71 ± 1	70 ± 2	69 ± 1	
Study II	Control	77 ± 1	81 ± 2	83 ± 2	80 ± 2
	Functional	75 ± 2	72 ± 2	70 ± 1	72 ± 2
	Nutraceutical	76 ± 2	72 ± 1	69 ± 1	72 ± 2
	Means	76 ± 1	75 ± 2	74 ± 2	
Study III	Control	93 ± 2	97 ± 2	104 ± 2	98 ± 1
	Functional	95 ± 2	90 ± 2	86 ± 1	90 ± 2
	Nutraceutical	94 ± 2	86 ± 1	82 ± 2	88 ± 2
	Means	94 ± 1	91 ± 2	91 ± 2	

[a] Study I: normal rats. Study II: hyperglycemic rats. Study III: hypercholesterolemic rats.

Table 6 Means for glucose level (mg dL^{-1}) of rats in different studies[a]

Studies	Diet	Study intervals (days) 0	28	56	Means
Study I	Control	92 ± 2	93 ± 2	94 ± 2	93 ± 2
	Functional	91 ± 2	91 ± 2	89 ± 2	90 ± 2
	Nutraceutical	93 ± 2	89 ± 2	89 ± 2	90 ± 2
	Means	92 ± 2	91 ± 2	91 ± 2	
Study II	Control	119 ± 3	124 ± 3	131 ± 3	125 ± 3
	Functional	118 ± 3	111 ± 3	106 ± 2	112 ± 3
	Nutraceutical	119 ± 3	109 ± 2	103 ± 3	110 ± 3
	Means	119 ± 3	115 ± 3	113 ± 3	
Study III	Control	97 ± 2	101 ± 2	107 ± 2	102 ± 2
	Functional	99 ± 2	97 ± 2	95 ± 2	97 ± 2
	Nutraceutical	98 ± 2	94 ± 2	92 ± 2	95 ± 2
	Means	98 ± 2	97 ± 2	98 ± 2	

[a] Study I: normal rats. Study II: hyperglycemic rats. Study III: hypercholesterolemic rats.

Table 7 Means for insulin level (μU mL^{-1}) of rats in different studies[a]

Studies	Diet	Study intervals (days) 0	28	56	Means
Study I	Control	9 ± 0.2	8 ± 0.1	9 ± 0.2	9 ± 0.1
	Functional	9 ± 0.2	9 ± 0.2	9 ± 0.2	9 ± 0.1
	Nutraceutical	9 ± 0.2	9 ± 0.2	10 ± 0.2	9 ± 0.1
	Means	9 ± 0.2	9 ± 0.2	9 ± 0.1	
Study II	Control	10 ± 0.2	10 ± 0.2	9 ± 0.2	10 ± 0.1
	Functional	10 ± 0.2	10 ± 0.2	10 ± 0.1	10 ± 0.2
	Nutraceutical	10 ± 0.2	11 ± 0.2	11 ± 0.2	11 ± 0.1
	Means	10 ± 0.2	10 ± 0.1	10 ± 0.2	
Study III	Control	9 ± 0.2	9 ± 0.2	9 ± 0.2	9 ± 0.2
	Functional	9 ± 0.2	9 ± 0.2	9 ± 0.1	9 ± 0.1
	Nutraceutical	9 ± 0.1	10 ± 0.2	10 ± 0.2	10 ± 0.1
	Means	9 ± 0.2	9 ± 0.2	9 ± 0.2	

[a] Study I: normal rats. Study II: hyperglycemic rats. Study III: hypercholesterolemic rats.

Table 8 Means for red blood cells (10^6 μL^{-1}) of rats in different studies[a]

Studies	Diet	Study intervals (days) 0	28	56	Means
Study I	Control	7 ± 0.2	8 ± 0.1	8 ± 0.2	8 ± 0.1
	Functional	7 ± 0.1	7 ± 0.1	7 ± 0.1	7 ± 0.2
	Nutraceutical	7 ± 0.2	7 ± 0.2	7 ± 0.2	7 ± 0.2
	Means	7 ± 0.1	7 ± 0.2	7 ± 0.2	
Study II	Control	8 ± 0.2	8 ± 0.1	9 ± 0.2	8 ± 0.2
	Functional	8 ± 0.2	8 ± 0.2	8 ± 0.2	8 ± 0.2
	Nutraceutical	8 ± 0.2	8 ± 0.1	8 ± 0.2	8 ± 0.2
	Means	8 ± 0.1	8 ± 0.2	8 ± 0.2	
Study III	Control	9 ± 0.2	9 ± 0.2	9 ± 0.2	9 ± 0.1
	Functional	9 ± 0.2	9 ± 0.2	9 ± 0.1	9 ± 0.2
	Nutraceutical	9 ± 0.2	9 ± 0.2	9 ± 0.2	9 ± 0.2
	Means	9 ± 0.2	9 ± 0.1	9 ± 0.2	

[a] Study I: normal rats. Study II: hyperglycemic rats. Study III: hypercholesterolemic rats.

Table 9 Means for white blood cells (10^3 μL^{-1}) of rats in different studies[a]

Studies	Diet	Study intervals (days) 0	28	56	Means
Study I	Control	10 ± 0.2	10 ± 0.2	10 ± 0.2	10 ± 0.2
	Functional	10 ± 0.2	10 ± 0.3	11 ± 0.3	10 ± 0.2
	Nutraceutical	10 ± 0.2	10 ± 0.2	11 ± 0.2	10 ± 0.2
	Means	10 ± 0.2	10 ± 0.2	11 ± 0.2	
Study II	Control	10 ± 0.2	10 ± 0.2	11 ± 0.3	10 ± 0.2
	Functional	10 ± 0.2	11 ± 0.3	11 ± 0.2	11 ± 0.2
	Nutraceutical	11 ± 0.2	11 ± 0.2	11 ± 0.1	11 ± 0.2
	Means	10 ± 0.2	11 ± 0.2	11 ± 0.3	
Study III	Control	10 ± 0.2	11 ± 0.2	11 ± 0.2	11 ± 0.2
	Functional	10 ± 0.1	11 ± 0.2	11 ± 0.2	11 ± 0.2
	Nutraceutical	10 ± 0.3	11 ± 0.3	12 ± 0.1	11 ± 0.3
	Means	10 ± 0.2	11 ± 0.1	11 ± 0.2	

[a] Study I: normal rats. Study II: hyperglycemic rats. Study III: hypercholesterolemic rats.

a maximum value for the control diet group (21.96 ± 0.56 g per rat per day), followed by the functional and nutraceutical groups with 21.22 ± 0.59 g per rat per day and 21.2 ± 0.61 g per rat per day, correspondingly. As regards study interval, feed intake values showed a significant increase from the 1st to 8th week for all diet groups. Similarly, study III showed the highest mean for the control group followed by the functional and nutraceutical group; values were recorded as 22.28 ± 0.63, 21.42 ± 0.58 and 21.38 ± 0.52 g per rat per day, correspondingly. Moreover, increasing time favored feed consumption and an overall increasing trend was observed in all groups with the highest rate in control groups in all studies.

According to Hininger-Favier *et al.*,[28] green tea intake led to lesser feed consumption in high fructose-fed rats compared to the control group. The feed intake in the control group after 6 weeks of study was recorded as 21.5 ± 0.6 g per rat per day. The feed intake in fructose-fed rats given 1 g tea solids per kg diet was recorded as 20.5 ± 0.9 g per rat per day while in rats given 2 g tea solids per kg diet it was recorded as 20.3 ± 1.5 g per rat per day, however, the difference was revealed to be non-significant.

Mean squares for water consumption illustrated a non-significant relation in the measured values with respect to treatments but a significant increase during the trial interval, in all diet groups. It can be observed from Fig. 3 that in study I, the highest intake was recorded for the control group (27.2 ± 0.77 mL per rat per day) and the lowest for the nutraceutical group (25.89 ± 0.64 mL per rat per day). Similarly for studies II and III, the maximum intake was noted for the control groups as 28.64 ± 0.81 and 29.18 ± 0.76 mL per rat per day, respectively, whilst the lowest was for the nutraceutical groups as 27.06 ± 0.58 and 27.93 ± 0.62 mL per rat per day. The results showed a significant increase in means for water intake from the 1st to 8th week irrespective of the treatments. The obtained data is also comparable to the efficacy results of Haque *et al.*[29] who incorporated green tea catechins in rodents and evaluated their impact on the learning capacity of subjects. They found insignificant difference among daily water intake values from all study groups *i.e.* control (27.7 ± 1.7 mL per rat per day), green tea catechins 0.1% (26.0 ± 1.4 mL per rat per day) and green tea catechins 0.5% (26.2 ± 1.0 mL per rat per day).

Body weight

Mean squares for rat body weights show a significant difference in body weights in relation to treatments and trial interval. Body weights of rats were measured once at the initiation of study and then weekly throughout the efficacy trial, data presented as graph (Fig. 4). At the start of trial, body weights for study I were noted as 131.28 ± 3.94, 133.72 ± 3.78 and 132.46 ± 2.85 g per rat for control, functional and nutraceutical diet groups, correspondingly. At the termination of the study, these values were recorded to be 227.12 ± 5.22, 212.77 ± 5.17 and 208.5 ± 4.32 g per rat, respectively. Means for body weights clearly show a higher weight gain in the control group compared to the other two groups. A similar increasing trend was observed for body

Table 10 Means for platelets (10^3 μL^{-1}) of rats in different studies[a]

Studies	Diet	Study intervals (days) 0	28	56	Means
Study I	Control	1033 ± 28	1026 ± 32	1050 ± 28	1036 ± 21
	Functional	1037 ± 22	1065 ± 23	1084 ± 34	1062 ± 32
	Nutraceutical	1041 ± 26	1082 ± 29	1116 ± 26	1079 ± 29
	Means	1037 ± 25	1057 ± 24	1083 ± 29	
Study II	Control	1068 ± 31	1075 ± 26	1109 ± 28	1084 ± 28
	Functional	1036 ± 25	1098 ± 33	1156 ± 31	1096 ± 33
	Nutraceutical	1041 ± 27	1104 ± 21	1191 ± 29	1112 ± 31
	Means	1048.33 ± 25	1092 ± 28	1152 ± 33	
Study III	Control	1054 ± 23	1076 ± 29	1090 ± 22	1073 ± 21
	Functional	1057 ± 27	1094 ± 26	1122 ± 31	1091 ± 27
	Nutraceutical	1062 ± 31	1105 ± 32	1142 ± 26	1103 ± 25
	Means	1057 ± 24	1091 ± 28	1118 ± 24	

[a] Study I: normal rats. Study II: hyperglycemic rats. Study III: hypercholesterolemic rats.

weights in study II over the trial interval. The values increased from 133.46 ± 2.91, 135.15 ± 3.54 and 134.59 ± 3.87 g per rat for control, functional and nutraceutical group, correspondingly and enhanced to 243.7 ± 6.02, 219.8 ± 5.79 and 218.5 ± 5.94 g per rat for the respective diet groups. The maximum weight gain was observed for the control group whilst the lowest weight gain was noted for the nutraceutical diet group. Similarly for study III (hypercholesterolemic rats), body weights recorded at the start of the trial were 132.84 ± 3.86, 134.66 ± 3.54 and 134.59 ± 2.91 g per rat for control, functional and nutraceutical groups, respectively. These values increased to 254.67 ± 6.29, 234.8 ± 5.88 and 230.83 ± 5.62 g per rat, correspondingly, at the end of efficacy trial. In all study groups, the maximum weight gain was associated with the control diet group while the nutraceutical diet group showed the lowest weight gain after eight weeks of *in vivo* experimentation.

The data obtained was comparable to previous *in vivo* biological studies. According to Uchiyama *et al.*,[30] mice fed on tea polyphenols showed slower gain in body weight during eight weeks of study. Ingestion of 5% tea polyphenol extract led to a decrease of 32.8% in body weight gain among normal rodents whilst the decrease was 44.2% among high-fat consuming subjects.

Serum lipid profile

Cholesterol. Green tea possesses hypocholesterolemic properties and can lower cholesterol levels in normal as well as hypercholesterolemic rats. Mean squares for serum lipid profile showed a significant difference in cholesterol levels in all three studies as a function of treatments. Results were also found to be significant with respect to interval and interaction of treatment and interval for study II and III but non-significant for study I. Means for cholesterol levels are presented in Table 2. In study I, highest value for cholesterol (82 ± 1 mg dL^{-1}) was measured in control diet group trailed along by functional (79 ± 1 mg dL^{-1}) and nutraceutical (79 ± 1 mg dL^{-1}) diet groups. During 56 days of trial, an increase in cholesterol level from 81 ± 2 to 82 ± 2 mg dL^{-1} was observed in control group. Diets containing green tea caused significant decrease in serum cholesterol levels of rats with a decrease from 80 ± 2 to 77 ± 1 mg dL^{-1} in the functional diet group while it was 81 ± 1 to 77 ± 1 mg dL^{-1} in the nutraceutical group. Results revealed a 4.11% reduction in cholesterol levels with the functional diet and a 5.57% reduction with the nutraceutical diet. Likewise, in study II the highest value (116 ± 3 mg dL^{-1}) was recorded for hyperglycemic rats from the control diet group followed by the functional (103 ± 2 mg dL^{-1}) and nutraceutical (103 ± 2 mg dL^{-1}) diet groups. During the study interval, the control group showed an increase in cholesterol level from 110 ± 3 to 121 ± 3 mg dL^{-1}. The functional diet showed a reduction from 98 ± 2 to 108 ± 2 mg dL^{-1} (9.35%) while the nutraceutical diet reduced cholesterol level from 111 ± 2 to 98 ± 2 mg dL^{-1} (11.65%) over 2 months. In the same manner, means from study III showed the maximum cholesterol level (137 ± 2 mg dL^{-1}) in the control group followed by the functional (122 ± 1 mg dL^{-1}) and nutraceutical (120 ± 2 mg dL^{-1}) groups. Over the study interval, a 12.15% reduction was observed with the functional diet from 130 ± 3 to 114 ± 2 mg dL^{-1} whilst a 15.45% reduction was recorded with the nutraceutical diet from 131 ± 2 to 110 ± 3 mg dL^{-1}. The reduction in serum cholesterol recorded in studies II and III from 0 to 56 days was highly significant ($p < 0.01$).

Most recently, Onuoha *et al.*[31] evaluated the consequence of green tea consumption against hypercholesterolemia and also showed that the value of cholesterol for a subject taking green tea were lower (174.2 ± 2.3 mg dL^{-1}) than the values for control (183.7 ± 0.7 mg dL^{-1}). Earlier, Wu *et al.*[16] examined the serum cholesterol levels of male mice dosed with 625, 1250 and 2500 mg kg^{-1} per day and observed significant decrease at all dosages. The maximum reduction (27%) was observed at a dose level of 2500 mg kg^{-1} per day over 28 days of trial.

High density lipoproteins (HDL)

Mean squares for serum HDL showed a non-significant difference in values for study I and II, however, in study III significant difference was observed regarding different treatments. No significant difference was observed with respect to interval and interaction of treatment and interval in all studies. Means for serum HDL levels are presented in Table 3. In study I, the highest value for HDL (35.61 ± 0.88 mg dL^{-1}) was measured for the nutraceutical diet group followed by the functional (34 ± 0.9 mg dL^{-1}) and control (34 ± 0.6 mg dL^{-1}) diet groups. During 56 days of the trial, a diet with green tea caused an increase in HDL levels of rats with an increase from 34 ± 0.6 to 34 ± 0.9 mg dL^{-1} in the functional diet group, while it was 35 ± 0.9 to 35 ± 0.5 mg dL^{-1} in nutraceutical group. Likewise, in study II the highest value (40 ± 0.9 mg dL^{-1}) was recorded for hyperglycemic rats from the nutraceutical diet group followed by the functional (39 ± 0.8 mg dL^{-1}) and control (39 ± 1 mg dL^{-1}) diet groups. During the study interval, the maximum increase was recorded for the nutraceutical diet as 39 ± 1 to 40 ± 1 mg dL^{-1} (2.35%) over 2 months.

For study III, mean HDL levels showed a maximum (49 ± 1 mg dL^{-1}) in the nutraceutical group followed by the functional (48 ± 1 mg dL^{-1}) and control (46 ± 1 mg dL^{-1}) groups. Over the study interval, a 3.12% increase was observed with the nutraceutical diet from 48 ± 1 to 49 ± 0.8 mg dL^{-1} whilst only a 2.08% increment was recorded with the functional diet from 47 ± 0.9 to 48 ± 1 mg dL^{-1}. The increase in serum HDL recorded in study III from 0 to 56 days was observed to be significant ($p < 0.05$) whilst control hyperglycemic and control hypercholesterolemic groups showed a decrease in insulin levels, indicating ailing conditions.

Moreover, Kasetti *et al.*[32] reported a decrease in high density lipoprotein (HDL) cholesterol in streptozotocin induced diabetic rats. Babu *et al.*[33] showed that green tea extract given to diabetic rats resulted in reduced cholesterol, triglyceride, free fatty acid and LDL levels, while increasing the serum HDL levels of rats. Moreover, they affirmed the antihyperglycemic and hypolipidemic activity of green tea extract.

Low density lipoproteins (LDL)

Green tea can reduce LDL cholesterol levels in normal as well as hypercholesterolemic rats. Mean squares for serum lipid profile

showed a significant difference in LDL levels in all three studies as a function of treatment, interval and their interaction. Means for LDL levels are given in Table 4. Study I contained the lowest value (30.82 ± 0.74 mg dL^{-1}) in the nutraceutical group while the highest (33 ± 0.4 mg dL^{-1}) in control diet group. During the 2 months of the trial, an increase in LDL level from 33.26 ± 0.81 to 33.89 ± 0.54 mg dL^{-1} was observed in the control group. Functional and nutraceutical diets resulted in significantly reduced LDL levels, with a decrease from 32 ± 0.6 to 30 ± 0.2 mg dL^{-1} through the functional diet and 32 ± 0.7 to 29 ± 0.6 mg dL^{-1} through the nutraceutical diet. Likewise, in study II the highest value (59 ± 1 mg dL^{-1}) was recorded for hyperglycemic rats from control diet group while functional (52 ± 1 mg dL^{-1}) and nutraceutical (51 ± 0.8 mg dL^{-1}) diet groups showed reduced levels. During the study interval, the control group showed an increase in LDL level from 55 ± 0.9 to 62 ± 1 mg dL^{-1}. The functional diet showed a reduction from 56 ± 1 to 48 ± 0.8 mg dL^{-1} (13.67%) while for the nutraceutical diet it was from 57 ± 1 to 47 ± 0.8 mg dL^{-1} (17.44%) over 2 months. In the same manner, means from study III showed elevated LDL (68 ± 1 mg dL^{-1}) in untreated hypercholesterolemic rats in contrast to lower levels in the functional (59 ± 1 mg dL^{-1}) and nutraceutical (56 ± 1 mg dL^{-1}) groups. Over the study interval, a 16.91% reduction was observed with the functional diet from 65 ± 1 to 54 ± 1 mg dL^{-1} whilst an appreciable 21.53% reduction was recorded with the nutraceutical diet from 63 ± 1 to 50 ± 1 mg dL^{-1}.

The results of the present project are comparable to earlier findings. Kim[21] and coworkers carried out a systematic review of various research findings and affirmed the link of green tea intake with reduced cholesterol and LDL levels. Nantz *et al.*[34] reported a significant decrease in LDL levels in men during 3 weeks of green tea ingestion in the form of encapsulated tea components of defined composition.

Triglycerides

The mean squares for serum lipid profile showed a significant effect of treatments and interval on triglycerides level in all three studies while there was a variable effect for interaction. Means presented in Table 5 indicate that study I had the maximum value (71 ± 2 mg dL^{-1}) in the control group while lower values in functional (70 ± 2 mg dL^{-1}) and nutraceutical (69 ± 1 mg dL^{-1}) diet groups. The 56 days of trial led to increased triglyceride levels from 71 ± 2 to 72 ± 1 mg dL^{-1} in the control group. Green tea-containing diets resulted in significantly reduced levels, 71 ± 2 to 68 ± 1 mg dL^{-1} with the functional diet and 71 ± 2 to 68 ± 2 mg dL^{-1} with the nutraceutical diet. The results revealed a 3.56 and 5.07% reduction with the functional and nutraceutical diets, respectively. Likewise, in study II the highest value (80 ± 2 mg dL^{-1}) was recorded for hyperglycemic rats from the control diet group while the functional (72 ± 2 mg dL^{-1}) and nutraceutical (72 ± 2 mg dL^{-1}) diet groups showed reduced levels. During the trial interval, the control group showed an increase in triglycerides from 77.42 ± 1.54 to 83 ± 2 mg dL^{-1}. The functional diet showed a reduction from 75 ± 2 to 70 ± 1 mg dL^{-1} (7.23%) while the nutraceutical diet gave the best results with a reduction from 76 ± 2 to 69 ± 1 mg dL^{-1} (9.96%) over 2 months. Means from study III showed the same trend with an elevated level (98 ± 1 mg dL^{-1}) in control hypercholesterolemic rats but lower levels in the functional (90 ± 2 mg dL^{-1}) and nutraceutical (88 ± 2 mg dL^{-1}) groups. Over the study interval, a 9.36% reduction was observed with the functional diet from 95 ± 2 to 86 ± 1 mg dL^{-1} whilst a greater reduction (12.92%) was recorded with the nutraceutical diet from 94 ± 2 to 82 ± 2 mg dL^{-1}.

The current data is in corroboration with previous research work, as Li *et al.*[35] expressed that green tea extract caused significant reduction in triglycerides level not only in plasma but also in liver and heart tissues in fructose-fed rodents. They reported triglyceride accumulation reduced by 42% in liver and 32.5% in heart tissues at a high dose of green tea supplementation *versus* control, incorporated in rats for 4 weeks.

Serum glucose and insulin analysis

Glucose. Green tea possesses anti-hyperglycemic properties and can help maintain elevated glucose levels in hyperglycemic rats. Mean squares for serum glucose and insulin levels showed a significant difference in glucose levels in all studies as a function of treatment. However, the results showed variable effects with respect to interval and the interaction of treatment and interval. Means for serum glucose are presented in Table 6. In study I, the highest value for glucose (93 ± 2 mg dL^{-1}) was measured in the control diet group, while the functional and nutraceutical diet groups showed lower levels, 90 ± 2 mg dL^{-1} and 90 ± 2 mg dL^{-1}, respectively. Two months of study led to an increase in the glucose level of control group subjects from 92 ± 2 to 94 ± 2 mg dL^{-1}. The functional diet showed a reduction from 91 ± 2 to 89 ± 2 mg dL^{-1} (2.37%) while the nutraceutical group reduced from 93 ± 2 to 89 ± 2 mg dL^{-1} (4.69%) over 2 months. Similarly, in study II the hyperglycemic control diet group showed an elevated glucose level (125 ± 3 mg dL^{-1}) while lower levels were observed in functional (112 ± 3 mg dL^{-1}) and nutraceutical (110 ± 3 mg dL^{-1}) diet groups. During the study interval, the control group showed increased serum glucose from 119 ± 3 mg dL^{-1} on 0 day to 131 ± 3 mg dL^{-1} after 56 days. The functional diet showed a reduction from 118 ± 3 to 106 ± 2 mg dL^{-1} (9.89%) while the nutraceutical diet reduced from 119 ± 3 to 103 ± 3 mg dL^{-1} (13.39%) over 2 months. Means from study III showed increased glucose level (102 ± 2 mg dL^{-1}) in the control hypercholesterolemic group followed by the functional diet group (97 ± 2 mg dL^{-1}), while the lowest value was recorded for the nutraceutical (95 ± 2 mg dL^{-1}) group. Over the study interval, a 3.85% reduction was observed with the functional diet from 99 ± 2 to 95 ± 2 mg dL^{-1} whilst a 6.03% reduction was recorded with the nutraceutical diet from 98 ± 2 to 92 ± 2 mg dL^{-1}, giving the best results.

The current results highly correlate with earlier research findings, affirming a decline in serum glucose levels with green tea intake. Tsuneki *et al.*[36] reported better glucose metabolism and significantly reduced blood glucose with the administration a suspension of green tea powder in human subjects. Moreover, they evaluated the antihyperglycemic properties of

green tea in diabetic mice and observed a significant decrease from 235 ± 15 mg dL^{-1} to 116 ± 12 mg dL^{-1} and 201 ± 10 mg dL^{-1} to 106 ± 8 mg dL^{-1} after 6 hours with two different green tea cultivars having 16.3 and 10.5 g total catechins per 100 g tea powder, respectively.

Insulin. Mean squares for serum insulin showed significant differences in insulin levels for study I, II and III as a function of treatment. No significant difference was observed with respect to interval and interaction of treatment and interval in all studies. It can be observed from means for serum insulin (Table 7) that in study I, the maximum value for insulin (9 ± 0.1 μU mL^{-1}) was measured for the nutraceutical diet group followed by the functional (9 ± 0.1 μU mL^{-1}) and control (9 ± 0.1 μU mL^{-1}) diet groups. During the trial interval, a diet with green tea caused non-significant elevation in insulin levels of rats with an increase from 9 ± 0.2 μU mL^{-1} to 9 ± 0.2 μU mL^{-1} in the functional diet group and 9 ± 0.2 μU mL^{-1} to 10 ± 0.2 μU mL^{-1} in the nutraceutical group. The results revealed a 2.19% increment with the functional diet and a 3.61% increase with the nutraceutical diet, higher than the 1.95% increase in the control group.

In study II the highest value (11 ± 0.1 μU mL^{-1}) was recorded for hyperglycemic rats from the nutraceutical diet group, followed by the functional (10 ± 0.2 μU mL^{-1}) and control (10 ± 0.1 μU mL^{-1}) diet groups. During the study interval, the maximum increase was recorded for the nutraceutical diet from 10 ± 0.2 μU mL^{-1} to 11 ± 0.2 μU mL^{-1} (7.06%) while the functional diet showed a smaller increment from 10 ± 0.2 μU mL^{-1} to 10 ± 0.1 μU mL^{-1} (4.47%) over 2 months. Means of serum insulin for study III showed a maximum value (10 ± 0.1 μU mL^{-1}) in the nutraceutical group followed by the functional (9 ± 0.1 μU mL^{-1}) and control (9 ± 0.2 μU mL^{-1}) groups. Over the study interval, a 4.89% increase was observed with the nutraceutical diet from 9 ± 0.1 μU mL^{-1} to 10 ± 0.2 μU mL^{-1} whilst only a 3.67% increment was recorded with the functional diet, from 9 ± 0.2 μU mL^{-1} to 9 ± 0.1 μU mL^{-1}. Control hyperglycemic and control hypercholesterolemic groups showed a decrease in insulin levels, indicating diseased conditions.

Recently, Li[37] and co-researchers carried out an experimental trial on rats to evaluate the effect of maternal green tea supplementation on alleviation of insulin resistance in male offspring. They reported a 57% reduction in insulin resistance along with improved glucose metabolism in offspring born to obese female rats administrated with green tea extract.

Hematological study

Blood hematological analysis was performed to get an estimation of green tea intake on red blood cell- (RBC), white blood cell- (WBC) and platelet count. Mean squares regarding these parameters showed non-significant differences throughout the efficacy trial as a function of treatment, interval and their interaction except for the effect of treatment in study I. Similarly, it can be observed that there was a non-significant effect of green tea on WBC and platelet count of rats with treatment and interval, as well as their interaction.

Means for RBC count (Table 8) showed a decrease with green tea administration in study I, II and III while a non-significant increase was observed in control group subjects. For study I, a lower RBC count (7 ± 0.2 × 10^6 μL^{-1}) was estimated in the nutraceutical group as compared with the functional (7 ± 0.2 × 10^6 μL^{-1}) and control (8 ± 0.1 × 10^6 μL^{-1}) groups. Study II showed the same trend with increase in RBC count for the control and reduction for green tea treated groups. The nutraceutical diet attained an RBC count of 8 ± 0.2 × 10^6 μL^{-1}, comparatively lower than the functional and control diets at 8 ± 0.2 and 8 ± 0.2 × 10^6 μL^{-1}, correspondingly. Likewise, study III showed values for the nutraceutical, functional and control groups as 9 ± 0.2, 9 ± 0.2 and 9 ± 0.1 × 10^6 μL^{-1}, respectively.

Means for WBC count are presented in Table 9, showing non-significant differences in all values. However in green tea treated diet groups (functional and nutraceutical), WBC count increased slightly as a function of time over 56 days, in all studies. Study I showed means for control, functional and nutraceutical groups of 10 ± 0.2, 10 ± 0.2 and 10 ± 0.2 × 10^3 μL^{-1}, respectively. In study II, the maximum count (11 ± 0.2 × 10^3 μL^{-1}) was observed in the nutraceutical group followed by the functional (11 ± 0.2 × 10^3 μL^{-1}) and control (10 ± 0.2 × 10^3 μL^{-1}) groups. Study III presented similar results with nutraceutical, functional and control groups showing values as 11 ± 0.3, 11 ± 0.2, 11 ± 0.2 × 10^3 μL^{-1}, respectively. Time interval showed a non-significant effect on the values observed in all studies.

Platelet count showed a slight increase with green tea treatments, means are shown in Table 10. In study I, the nutraceutical group showed the highest count (1079 ± 29 × 10^3 μL^{-1}) followed by the functional (1062 ± 32 × 10^3 μL^{-1}) and control (1036 ± 21 × 10^3 μL^{-1}) groups. Likewise, in study II, the maximum count (1112 ± 31 × 10^3 μL^{-1}) was observed in the nutraceutical group followed by the functional (1096 ± 33 × 10^3 μL^{-1}) and control (1084 ± 28 × 10^3 μL^{-1}) groups. Study III presented similar results with the nutraceutical, functional and control groups showing values of 1103 ± 25, 1091 ± 27, 1073 ± 21 × 10^3 μL^{-1}, respectively. All study groups showed non-significant differences in the platelet count during the two months of the trial.

The current data with a non-significant effect of green tea on hematological parameters is highly consistent with previous findings. Isbrucker *et al.*[38] observed a non-significant effect of EGCG on WBC and platelet count, however, some significant results were observed with RBC count at lower dosage. However, the phenomenon of reduction in RBC count is not fully understood and needs further investigation.[39] Takami[40] and coworkers evaluated the toxicity of green tea catechins and carried out complete hematological analysis for the purpose. They found a slight decrease in RBC but an increase in WBC and platelet count in catechins-fed rats over a period of 90 days. However, all the differences were statistically non-significant. Wang *et al.*[41] studied toxicity evaluation of green tea extract in normal mice and demonstrated no significant effects of green tea extract on the values of RBC, WBC and platelet count.

Conclusion

Cultivated across more than 30 states with 2.7 million hectares globally, green tea has received significant attention, both in

scientific and consumer communities, owing to its health benefits against a variety of maladies ranging from weight loss to cancer. The present project was an attempt to explore the functional/nutraceutical role of green tea against hyperglycemia and hypercholesterolemia. For this purpose, three types of studies were carried out on the basis of diets *i.e.* study I (normal diet), study II (high sucrose diet), study III (high cholesterol diet), in Sprague Dawley rats. A decline was observed in the levels of cholesterol, LDL, triglycerides and glucose compared to controls after the consumption of functional and nutraceutical diets. It was observed that reduction of cholesterol, LDL, triglycerides and glucose was more in groups fed on nutraceutical diets while it was lower in the functional diet group, followed by the normal group. It was also depicted that level of insulin and HDL increased in the functional and nutraceutical diet groups. Regarding hematological analysis, treatment has a non-significant effected on all hematological parameters, however, with passage of time red blood cells and white blood cells changed significantly in study I and II. Conclusively, green tea has been proved to hold functional/nutraceutical worth against various lifestyle related threats. Diets containing green tea active ingredients as an adjunct, not only attenuated hyperglycemia and hypercholesterolemia in rodent subjects but also improved antioxidant status when used in food products.

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