PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 9 (3): 222-229, 2010
ISSN 1680-5194

Safety Assessment of Functional Drinks Prepard From Green Tea Catechins and Epigallocatechin Gallate

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Abstract: Increasing awareness regarding natural ingredients has led to utilization of functional beverages in diet based therapy. Present project was designed to evaluate safe use of functional drink prepared from green tea active ingredients. Efficacy trial was conducted in male Sprague Dawley rats for period of eight weeks. Functional drinks were prepared by adding catechins and Epigallocatechin Gallate (EGCG) @ 550 mg/500 mL and provided to rats for the period of eight weeks. Four types of studies were conducted consisting of different types of diets i.e. study I (normal diet), study II (high cholesterol diet), study III (high sucrose diet), study IV (high cholesterol+high sucrose diet). The results revealed safety of functional drinks as values for liver and kidney function tests and serum proteins remained in normal range. Organs to body weight ratio were non-significantly effected by functional drinks. Conclusively it can be suggested that functional drinks carrying green tea catechins and EGCG are safe and could be a part of diet therapy for treatments of lifestyle related disorders.

Key words: Functional drink, safety, green tea, liver, catechins, EGCG

INTRODUCTION

Recently, pivotal linkages ascertained between health and nutrition has turned away the human inclination towards plant based natural products to treat various disorders. In this milieu, green tea has gained popularity because of its health enhancing prospective. Green tea was accidentally discovered by Shen Nung a Chinese emperor in 2737 B.C (Wheeler and Wheeler, 2004) and is one of most widely consumed beverages in Asian countries (Zaveri, 2006). Tea is grown over 30 countries (Graham, 1992) and occupies about 2.7 million hectares of cultivable area of the world (Mondal *et al*., 2004). China, Japan, Taiwan, India, Bangladesh, Sri Lanka and Kenya are the major producers (Shaheen *et al*., 2006). Worldwide per capita consumption of tea is 40 L per year (Vinson *et al*., 2004), approximately 3 million metric tons of tea is produced annually, increasing at rate of 2.1% (Yang and Landau, 2000).

Polyphenols are the main constituents of green tea, accounting for 25-35% on dry weight basis (Balentine *et al*., 1997; Shaheen *et al*., 2006; Yao *et al*., 2006). Health claims of green tea are attributed to its polyphenolic fractions known as catechins, including Epicatechins (EC), Epicatechin Gallate (ECG), Epigallocatechin (EGC) and Epigallocatechin Gallate (EGCG). Among catechins, EGCG is the most promising component (Demeule *et al*., 2002; Kovacs *et al*., 2004; Bettuzzi *et al*., 2006; Wang *et al*., 2006) constituting 48-55% of total polyphenols (Ho *et al*., 1997) and is responsible for majority of the health benefits of green tea (Nagle *et al*., 2000; Lambert and Yang, 2003; Wolfram *et al*., 2006). The chemical composition of tea varies with the growing conditions like climate, season, agricultural practices, variety, age and position of the leaf (Katiyar and Mukhtar, 1996a,b; Aherne and O'Brien, 2002; Fernandez *et al*., 2002; Lin *et al*., 2003). In green tea, catechins are present in higher amounts than that of black or oolong tea, because of the processing differences (Zaveri, 2006). Some other sources of catechins are red wine, fruits like plum, apples, peach, strawberry, cherry, broad bean, lentil and cocoa (Scalbert *et al*., 2005; Yilmaz, 2006).

Green tea possesses antioxidative (Yoshino *et al*., 1994; Miura *et al*., 2001; Hakim *et al*., 2003; Suzuki *et al*., 2004), antiallergic (Sano *et al*., 1999), anti-inflammatory (Dona *et al*., 2003; Lee *et al*., 2005) and hypolipidemic (Yoshino *et al*., 1994; Imai and Nakachi, 1995; Murase *et al*., 2002; Raederstorff *et al*., 2003; Zheng *et al*., 2004) properties.

Diets rich in cholesterol lead to higher production of Reactive Oxygen Species (ROS) resulting in oxidative stress. ROS attack polyunsaturated fatty acids in cell membrane resulting in lipid peroxidation products leading to structural and functional cell damage (Kuper *et al*., 2000). The levels of ALT, ALP, AST and bilirubin are altered thereby damaged structural integrity of the liver, as they are present in cytoplasm and are released in blood circulation after cellular damage (Recknagel *et al*., 1989; Dobrzynska *et al*., 2004).

MATERIALS AND METHODS

Present research project was conducted in the Postgraduate Research Laboratory, National Institute of

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Food Science and Technology (NIFSAT). Green tea leaves of Qi-Men variety were obtained from 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). Sprague Dawley rats used in the efficacy trials were acquired from National Institute of Health (NIH) Islamabad. Diagnostic Kits used were from Sigma-Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia).

Safety assessment of functional drink: Functional drinks (T_0, T_1, T_2) were prepared by adding catechins and Epigallocatechin Gallate (EGCG) @ 550 mg/500 mL in respective drink and a control was also prepared for comparison purpose.

Experimental animals and housing conditions: One hundred and twenty male Sprague Dawley rats (seven weeks old) weighing 125±10 g were procured from National Institute of Health (NIH), Islamabad and housed in the Animal Room of National Institute of Food Science and Technology. The animals were acclimatized by feeding basal diet (AIN-76A) for a period of one week. The temperature (23±2°C) and relative humidity (55±5%) were maintained throughout the experiment period with 12 h light-dark period.

Experimental design: After one week of wash out period rats were divided into four groups according to four different types of diet i.e. normal diet, high cholesterol diet, high sucrose diet and high cholesterol + high sucrose diet. In each group rats were further divided into three subgroups (Table 1). Functional drink was provided in polypropylene bottles with stainless steel sipper tubes. The experimental diets comprised of corn oil (10%), protein (10%), corn starch (66%) and cellulose (10%), mineral (3%) and vitamin mixture (1%). In high cholesterol diet and sucrose diets, cholesterol and sucrose were added at 1 and 40%, respectively. The overnight fasted rats were sacrificed after eight weeks of feeding with simultaneous intake of functional drinks. Body organs including heart, liver, left and right kidney, spleen, lungs and pancreas were weighed to calculate organ to body weigh ratio. Blood samples of rats were collected through cardiac puncture; EDTA coated tubes were employed for serum collection and further used to perform various assays through Microlab-300, Merck, Germany.

Organs weight: Organs i.e. liver, heart, kidney, spleen, lungs and pancreas were collected after dissection to determine the effect of test diets on organ weights of rats. The organs were properly cleaned and weighed on electronic balance (Dyer *et al*., 2008). The results were expressed as organ to body weight ratios (g/100 g of body weight).

Table 1: Diet plan used in the studies

Study No.	Rats groups	Drinks
Normal diet (I)	1	T_0
	2	T_1
	3	T_2
High cholesterol diet (II)	1	T_0
	2	T_1
	3	T_2
High sucrose diet (III)	1	T_0
	2	T_1
	3	T_2
High cholesterol +	1	T_0
high sucrose diet (IV)	2	T_1
	3	T_2

Liver and renal function tests: Liver function tests including Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) and bilirubin total were assessed. Levels of AST and ALT were measured by the Dinitrophenylhydrazene (DNPH) method using Sigma Kits 59-50 and 58-50, respectively and ALP by Alkaline Phosphates-DGKC method (Thomas, 1998; Moss and Handerson, 1999). Bilirubin total was determined by Jendrassik-Grof method (Tolman and Rej, 1999). The serum urea (GLDH-method) and creatinine (Jaffe-method) were determined using commercial kits (Jacobs *et al*., 1996; Thomas, 1998) to assess the renal functionality of different rats groups in each study.

Serum proteins: Serum total proteins, albumins, globulin and A/G ratio were estimated using respective kits of Sigma-Aldrich Chemicals Co. (Bradford, 1976).

Statistical analysis: Completely Randomized Design (CRD) was applied and resultant data was subjected to statistical analysis using Cohort version 6.1 (Costat-2003). Analysis of Variance technique (ANOVA) was used to determine the level of significance (Steel *et al*., 1997).

RESULTS

Organs to body weight ratio: Organs weight was non-momentously affected by functional drinks in all studies (Table 2). Means for heart to body weight ratio of rats in different studies ranged from 0.32±0.03 to 0.44±0.04 g/100 g body weight. Likewise, non-momentous effect for liver weight was noted that ranged from 4.01±0.04 to 4.57±0.03 g/100 g body weight. Similarly, weight of right and left kidney of rats in different studies was affected non-significantly by functional drinks i.e. 0.40±0.03 to 0.46±0.02 and 0.38±0.02 to 0.47±0.03 g/100 g body weight, respectively. Spleen weight varied non-substantially from 0.31±0.03 to 0.36±0.03 g/100 g body

Table 2: Organs to body weight ratio (g/100 g body weight)

		Studies			
Parameters	Treatments	Study I	Study II	Study III	Study IV
Heart	T_0	0.34±0.02^{NS}	0.35±0.03^{NS}	0.36±0.03^{NS}	0.41±0.04^{NS}
	T_1	0.35±0.03^{NS}	0.38±0.02^{NS}	0.40±0.03^{NS}	0.44±0.04^{NS}
	T_2	0.32±0.03^{NS}	0.37±0.03^{NS}	0.38±0.02^{NS}	0.42±0.02^{NS}
Liver	T_0	4.01±0.04^{NS}	4.51±0.04^{NS}	4.52±0.03^{NS}	4.57±0.03^{NS}
	T_1	4.17±0.03^{NS}	4.32±0.04^{NS}	4.30±0.03^{NS}	4.33±0.04^{NS}
	T_2	4.20±0.03^{NS}	4.15±0.03^{NS}	4.14±0.04^{NS}	4.20±0.03^{NS}
Right kidney	T_0	0.43±0.04^{NS}	0.44±0.03^{NS}	0.44±0.04^{NS}	0.45±0.04^{NS}
	T_1	0.46±0.02^{NS}	0.42±0.04^{NS}	0.41±0.04^{NS}	0.42±0.02^{NS}
	T_2	0.46±0.02^{NS}	0.42±0.04^{NS}	0.40±0.03^{NS}	0.43±0.03^{NS}
Left kidney	T_0	0.43±0.03^{NS}	0.41±0.04^{NS}	0.43±0.04^{NS}	0.45±0.02^{NS}
	T_1	0.47±0.03^{NS}	0.41±0.04^{NS}	0.39±0.03^{NS}	0.38±0.02^{NS}
	T_2	0.46±0.03^{NS}	0.42±0.04^{NS}	0.40±0.02^{NS}	0.40±0.03^{NS}
Spleen	T_0	0.36±0.02^{NS}	0.34±0.02^{NS}	0.35±0.03^{NS}	0.36±0.03^{NS}
	T_1	0.35±0.03^{NS}	0.32±0.02^{NS}	0.31±0.03^{NS}	0.31±0.03^{NS}
	T_2	0.36±0.03^{NS}	0.32±0.02^{NS}	0.34±0.02^{NS}	0.32±0.03^{NS}
Lungs	T_0	1.16±0.10^{NS}	1.14±0.09^{NS}	1.15±0.09^{NS}	1.18±0.08^{NS}
	T_1	1.19±0.07^{NS}	1.10±0.06^{NS}	1.09±0.01^{NS}	1.12±0.08^{NS}
	T_2	1.19±0.09^{NS}	1.09±0.10^{NS}	1.10±0.10^{NS}	1.13±0.06^{NS}
Pancreas	T_0	0.60±0.05^{NS}	0.58±0.04^{NS}	0.58±0.03^{NS}	0.61±0.05^{NS}
	T_1	0.61±0.05^{NS}	0.56±0.04^{NS}	0.55±0.03^{NS}	0.59±0.04^{NS}
	T_2	0.61±0.05^{NS}	0.55±0.04^{NS}	0.56±0.03^{NS}	0.57±0.04^{NS}

weight in different studies. Mean values for lungs ranged from 1.09±0.01 to 1.19±0.09 g/100 g body weight in the entire efficacy trial. Means pertaining to the pancreas to body weight ratio were 0.55±0.03 to 0.61±0.05 g/100 g.

Liver and kidney functioning tests: Alanine Transaminase (ALT) values were non-significantly affected by functional drinks in T_0, T_1 and T_2 groups in study I. However in study II, higher ALT value (52.32±3.72 IU/L) was noted in T_0 group consuming control drink that reduced in T_1 (42.57±2.44 IU/L) and T_2 (41.33±2.12 IU/L) groups taking functional drinks. Likewise in study III, mean for ALT in T_0 was 48.09±2.25 IU/L that decreased to 42.57±1.44 IU/L in T_1 and 43.14±2.26 IU/L in T_2. In study IV, mean for T_0 was 54.28±3.93 IU/L whereas T_1 and T_2 groups provided functional drink showed significant reduction in ALT level i.e. 46.55±2.76 and 45.07±3.41 IU/L, respectively (Table 3).

Mean AST values for T_0, T_1 and T_2 groups in study I were 122.29±4.44, 125.66±5.83 and 121.46±5.89 IU/L, respectively. Means pertaining to AST level in study II, showed high value in T_0 (176.29±9.34 IU/L) as compared to T_1 (133.22±6.39 IU/L) and T_2 (130.85±6.24 IU/L). High sucrose diet (study III) given to rats resulted in elevated AST level in T_0 (149.10±7.20 IU/L) group provided drink without any active ingredients whilst its level was comparatively low in T_1 (132.54±5.32 IU/L) and T_2 (134.71±7.60 IU/L) groups consuming enriched functional drinks. Likewise in study IV, AST value for T_0 was 186.56±9.52 IU/L followed by T_1 and T_2 groups having mean values 137.56±8.86 and 128.39±5.01 IU/L, respectively for this trait.

Mean ALP values in study I (normal diet) for T_0, T_1 and T_2 groups were 169.60±8.47, 168.25±10.13 and 170.72±7.58 IU/L, respectively. In study II, supply of high cholesterol diet to rats lifted their ALP level to 266.14±7.69 IU/L in T_0 group consuming control drink whereas its value was decreased in T_1 and T_2 groups consuming catechins and EGCG enriched drinks to 203.86±6.10 and 196.51±8.34 IU/L, respectively. Likewise in study III, higher ALP value was recorded in T_0 (255.00±9.53 IU/L) as compared to T_1 (192.45±8.75 IU/L) and T_2 (195.46±9.24 IU/L) groups. Mean ALP value in study IV for T_0 group was 306.63±11.90 followed by T_1 (235.61±7.86 IU/L) and T_2 (234.79±6.91 IU/L) groups.

Mean values in T_0, T_1 and T_2 groups for bilirubin in study I were 0.71±0.03, 0.72±0.04 and 0.74±0.05 mg/dL, respectively. However in study II, higher bilirubin value (1.21±0.09 mg/dL) noted in T_0 group was reduced in T_1 (0.89±0.07 mg/dL) and T_2 (1.03±0.08 mg/dL) groups consuming functional drinks. Likewise in study III, bilirubin in T_0 was 1.02±0.07 mg/dL that decreased to 0.69±0.04 mg/dL in T_1 and 0.73±0.06 mg/dL in T_2. In study IV, mean for bilirubin in T_0 was 1.26±0.08 mg/dL whereas T_1 and T_2 groups showed significant reduction i.e. 0.79±0.05 and 0.96±0.08 mg/dL, respectively.

In study I, mean values for urea in T_0, T_1 and T_2 groups were 25.72±1.72, 24.93±0.86 and 25.41±1.43 mg/dL, respectively. In study II, there was noted high urea level 33.98±1.52 mg/dL in T_0 group that reduced to 26.85±0.50 mg/dL in T_1 and 28.73±1.18 mg/dL in T_2 group with concurrent intake of functional drinks containing active ingredients. Similarly in study III, rats showed uplifted urea level (30.70±2.18 mg/dL) in T_0

Table 3: Effect of functional drinks on liver and kidney functioning in different studies

		Studies			
Parameters	Treatments	Study I	Study II	Study III	Study IV
ALT (IU/L)	T_0	42.04±1.24^{NS}	52.32±3.72^{NS}	48.09±2.25^{NS}	54.28±3.93^{NS}
	T_1	42.85±2.52^{NS}	42.57±2.44**	42.57±1.44**	46.55±2.76**
	T_2	41.50±1.76^{NS}	41.33±2.12**	43.14±2.26**	45.07±3.41**
AST (IU/L)	T_0	122.29±4.44^{NS}	176.29±9.34^{NS}	149.10±7.20^{NS}	186.56±9.52^{NS}
	T_1	125.66±5.83^{NS}	133.22±6.39**	132.54±5.32*	137.56±8.86**
	T_2	121.46±5.89^{NS}	130.85±6.24**	134.71±7.60*	128.39±5.01**
ALP (IU/L)	T_0	169.60±8.47^{NS}	266.14±7.69^{NS}	255.00±9.53^{NS}	306.63±11.90^{NS}
	T_1	168.25±10.13^{NS}	203.86±6.10**	192.45±8.75**	235.61±7.86**
	T_2	170.72±7.58^{NS}	196.51±8.34**	195.46±9.24**	234.79±6.91**
Bilirubin (mg/dL)	T_0	0.71±0.03^{NS}	1.21±0.09^{NS}	1.02±0.07^{NS}	1.26±0.08^{NS}
	T_1	0.72±0.04^{NS}	0.89±0.07**	0.69±0.04**	0.79±0.05**
	T_2	0.74±0.05^{NS}	1.03±0.08**	0.73±0.06**	0.96±0.08**
Urea (mg/dL)	T_0	25.72±1.72^{NS}	33.98±1.52^{NS}	30.70±2.18^{NS}	38.26±1.96^{NS}
	T_1	24.93±0.86^{NS}	26.85±0.50**	27.03±1.52**	31.92±2.58**
	T_2	25.41±1.43^{NS}	28.73±1.18**	25.76±0.85**	34.33±2.80**
Creatinine (mg/dL)	T_0	0.81±0.05^{NS}	1.16±0.09^{NS}	0.99±0.07^{NS}	1.25±0.09^{NS}
	T_1	0.78±0.04^{NS}	0.82±0.05**	0.76±0.04**	0.87±0.06**
	T_2	0.80±0.05^{NS}	0.97±0.06**	0.85±0.05**	0.94±0.07**

group whereas its level reduced to 27.03±1.52 and 25.76±0.85 mg/dL in T_1 and T_2 groups, respectively. Maximum urea was in T_0 group (38.26±1.96 mg/dL) followed by T_1 (31.92±2.58 mg/dL) and T_2 (34.33±2.80 mg/dL) in study IV.

In study I, mean values for creatinine were 0.81±0.05, 0.78±0.04 and 0.80±0.05 mg/dL for T_0, T_1 and T_2 groups, respectively. Likewise in study II, means for creatinine in T_0 was 1.16±0.09 mg/dL followed by significant reduction in T_1 (0.82±0.05 mg/dL) and T_2 (0.97±0.06 mg/dL). In study III comprising of high sucrose diet, T_0 showed highest creatinine level (0.99±0.07 mg/dL) that momentously decreased to 0.76±0.04 and 0.85±0.05 mg/dL in T_1 and T_2 groups, respectively. Considering the results of study IV, maximum creatinine 1.25±0.09 mg/dL was recorded in T_0 group (control drink) that significantly reduced to 0.87±0.06 mg/dL in T_1 (drink containing catechins) and 0.94±0.07 mg/dL in T_2 (drink containing EGCG) groups.

Serum proteins: Serum proteins include total proteins, albumin, globulins and A/G ratio were estimated to establish safety of product (Table 4).

In study I, value for total proteins were 6.48±0.33, 6.29±0.49 and 6.40±0.02 g/dL in T_0, T_1 and T_2 groups, respectively. Likewise in study II, level of total proteins was 6.82±0.56 g/dL in T_0, 7.02±0.57 g/dL in T_1 and 6.85±0.34 g/dL in T_2. In study III, protein values for T_0, T_1 and T_2 groups were 7.26±0.30, 7.09±0.48 and 7.20±0.49 g/dL whereas 7.06±0.58, 7.38±0.42 and 7.35±0.31 g/dL, respectively in study IV.

In study I, mean albumin values were 3.18±0.24, 3.11±0.22 and 3.12±0.15 g/dL in T_0, T_1 and T_2 groups, respectively. Albumin level for T_0 group in study II was 3.05±0.14 g/dL that raised significantly in T_1 and T_2 groups to 3.86±0.11 and 3.62±0.25 g/dL, respectively. In study III, albumin values for T_0, T_1 and T_2 groups were 3.52±0.27, 3.52±0.13 and 3.46±0.29 g/dL, respectively. In study IV, albumin level (3.17±0.12 g/dL) in T_0 group was comparatively lower than T_1 (3.85±0.20 g/dL) and T_2 (3.70±0.21 g/dL) groups consuming functional drinks.

In study I, mean values for globulin were 2.79±0.13, 2.71±0.17 and 2.80±0.15 g/dL for T_0, T_1 and T_2 groups, respectively. Globulin level for T_0 group in study II was 3.25±0.24 g/dL that reduced momentously in T_1 and T_2 groups to 2.65±0.16 and 2.72±0.13 g/dL, respectively. In study III groups T_0, T_1 and T_2 showed globulin level of 3.20±0.22, 3.03±0.26 and 3.17±0.28 g/dL, respectively. Likewise in study IV, globulin level for T_0, T_1 and T_2 groups was 3.29±0.22, 2.95±0.15 and 3.02±0.25 g/dL, correspondingly.

Mean values for A/G ratio for T_0, T_1 and T_2 groups in study I were 1.14±0.07, 1.15±0.09 and 1.12±0.06, respectively. In study II, A/G ratio in T_0 group was 0.95±0.04 that momentously increased to 1.47±0.05 in T_1 and 1.34±0.02 in T_2 groups. In study III, mean values for A/G ratio were 1.12±0.05, 1.18±0.04 and 1.10±0.06 for T_0, T_1 and T_2 groups, respectively. Likewise in study IV, A/G ratio was 0.98±0.07 in T_0 group that substantially increased to 1.32±0.09 in T_1 and 1.23±0.07 in T_2.

DISCUSSION

Morita *et al.* (2009) delineated non-substantial effect of different green tea doses on rats organs weight like heart spleen and brain except for liver and kidney of rats. Likewise, Chengelis *et al.* (2008) mentioned that rat's organs like liver, kidneys, heart and spleen were not affected significantly by orally given green tea catechins up to dose of 2000 mg/kg/day for 28 days. In a research study Takami *et al.* (2008) reported similar non-momentous effect of green tea catechins (1.25%) on lungs, heart, spleen, liver and kidneys of rats. The

Table 4: Effect of functional drinks on serum proteins

		Studies			
Parameters	Treatments	Study I	Study II	Study III	Study IV
Total proteins (g/dL)	T_0	6.48±0.33^{NS}	6.82±0.56^{NS}	7.26±0.30^{NS}	7.06±0.58^{NS}
	T_1	6.29±0.49^{NS}	7.02±0.57^{NS}	7.09±0.48^{NS}	7.38±0.42^{NS}
	T_2	6.40±0.02^{NS}	6.85±0.34^{NS}	7.20±0.49^{NS}	7.35±0.31^{NS}
Albumin (g/dL)	T_0	3.18±0.24^{NS}	3.05±0.14^{NS}	3.52±0.27^{NS}	3.17±0.12^{NS}
	T_1	3.11±0.22^{NS}	3.86±0.11**	3.52±0.13^{NS}	3.85±0.20*
	T_2	3.12±0.15^{NS}	3.62±0.25**	3.46±0.29^{NS}	3.70±0.21*
Globulin (g/dL)	T_0	2.79±0.13^{NS}	3.25±0.24^{NS}	3.20±0.22^{NS}	3.29±0.22^{NS}
	T_1	2.71±0.17^{NS}	2.65±0.16**	3.03±0.26^{NS}	2.95±0.15^{NS}
	T_2	2.80±0.15^{NS}	2.72±0.13**	3.17±0.28^{NS}	3.02±0.25^{NS}
A/G ratio	T_0	1.14±0.07^{NS}	0.95±0.04^{NS}	1.12±0.05^{NS}	0.98±0.07^{NS}
	T_1	1.15±0.09^{NS}	1.47±0.05**	1.18±0.04^{NS}	1.32±0.09**
	T_2	1.12±0.06^{NS}	1.34±0.02**	1.10±0.06^{NS}	1.23±0.07**

results regarding organ to body weight ratio showed that green tea did not impart any hazardous effect on these organs as the values were within normal ranges proving the safe use of functional drink.

Liver is an important organ for the detoxification and deposition of endogenous and exogenous substances. ALT and AST are important serum enzymes as their varied concentrations indicate liver dysfunctioning (Wang *et al*., 2007). A number of natural/herbal products used against liver injury possess one or combination of antioxidant, antifibrotic, immune modulatory or antiviral activities (Seeff *et al*., 2001; Lee and Jeong, 2002; Shin *et al*., 2006). Recently, Noori *et al*. (2009) investigated the effect of green tea against carbon tetrachloride (CCL_4) induced liver cirrhosis in rodents modeling. Plasma Alanine Aminotransferase (ALT) was much lower in orally treated green tea group confirming its vitality against liver dysfunctions. Yasuda *et al*. (2009) revealed that 0.1% solution of EGCG in drinking water decreases serum AST and ALT raised by CCL_4 in rat modeling thus cures liver complications. Bose *et al*. (2008) also illustrated that ALT concentrations were reduced in EGCG treated high fat diet obese mice group. Likewise, Kuzu *et al*. (2008) mentioned that EGCG administration to Sprague Dawley rats for six weeks along with High Fat Diet (HFD) caused significant reduction in plasma ALT.

Feillet-Coudray *et al*. (2009) elucidated that diet rich in sucrose and fat leads to increased lipid peroxidation products resulting in oxidative stress however, green tea provides protection against oxidative damage thereby lowering Aspartate Aminotransferase (AST) activity (Panza *et al*., 2008). Earlier, Hassan *et al*. (2007) used carbon tetrachloride induced hepatotoxic rats to evaluate the protective role of green tea. They reported reduction in liver AST level with green tea.

In diabetic rats, treatment of green tea extract (300 mg/kgbodyweight/day) significantly lowers serum AST, proving its worth as therapeutic agent in diabetes complications (Babu *et al*., 2007). EGCG is an effective antioxidant (Yin *et al*., 2008) and its 0.1% solution decreases serum AST and ALT raised by CCL_4 in rat modeling, thus acts as remedy for liver complications (Yasuda *et al*., 2009).

Ramesh *et al*. (2009) determined the role of tea catechins in rats with hepatic oxidative abnormalities and highlighted that intraperitoneal injection of tea catechins decreased activities of serum AST, ALT and ALP.

Increased serum activities of total bilirubin, AST, ALP and ALT reveal cellular leakage and loss of functional integrity of cell membrane in liver (Mukherjee, 2003). The reduction in their levels confirms stabilization of plasma membranes as well as restoration of hepatic tissue damage (Lin *et al*., 2008). Recently, Morita *et al*. (2009) reported non-momentous effect of green tea catechins on serum chemistry including bilirubin. Likewise, Chengelis *et al*. (2008) also affirmed the safety issues of green tea catechins using up to 2000mg/kg/day; reported non-significant effect on markers of liver toxicity including AST, ALP, ALT and bilirubin.

High serum urea and creatinine concentrations reflect abnormal kidney functioning (Kataya and Hamza, 2008). Renno *et al*. (2008) proved the ability of tea catechins to normalize elevated level of urea. They mentioned significantly high urea nitrogen in serum of diabetic Sprague Dawley rats that reduced substantially by provision of green tea. In present findings though effect of functional drinks was significant on serum urea of rats provided cholesterol and sucrose rich diets but values were within normal limits.

The work of Sabu *et al*. (2002) supported the present finding of reduced creatinine by function drinks as they recorded significant reduction in serum creatinine level of diabetic rats by administration of green tea polyphenols. Likewise, Renno *et al*. (2008) observed similar declining trend in serum creatinine level by use of green tea solution as drinking source in diabetic rats. Morita *et al*. (2009) reported non-significant effect of green tea catechins up to dose of 1200 mg/kg/day on serum total proteins of Sprague Dawley rats. Likewise, Kao *et al*. (2000) expounded non-momentous effect of green tea epigallocatechin gallate on this trait.

Chengelis *et al.* (2008) mentioned non-momentous effect on serum albumin content of male and female rats during 28 days study period. Malley *et al.* (2007) reported the values i.e. 6.8-7.9, 3.8-4.2, 3.1-3.7 g/dL for total proteins, albumin and globulin, respectively that are in line with instant findings as serum albumin was in range from 3.05±0.34 to 3.86±0.61 g/dL. In present investigation though albumin level was increased momentously in study II and IV but values were within normal range.

In present investigation effect of functional ingredient on serum globulin was non-substantial except for study II in which declining trend was recorded nonetheless, values were within safe limit.

From present exploration it is observed that values for liver and kidney functioning tests were within normal range showing the acceptability of product. Moreover, protein related parameters showed non-momentous differences though some of values behaved substantially but were within normal range proving the functional worth of prepared drinks. Considering above all results, it is concluded that functional drinks are risk free and could be used against various ailments.

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