

Modulatory effects of black v. green tea aqueous extract on hyperglycaemia, hyperlipidaemia and liver dysfunction in diabetic and obese rat models

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Cardiovascular complications are a major cause of morbidity and mortality in patients with diabetes, obesity and the metabolic syndrome. Recently, there has been an increasing interest in tea as a protective agent against CVD. Here, we compared the modulatory effects of two different doses (50 and 100 mg/kg body weight given orally for 28 consecutive days) of black tea aqueous extract (BTE, rich in theaflavins and thearubigins) and green tea aqueous extract (GTE, rich in catechins) on experimentally induced hyperglycaemia, hyperlipidaemia and liver dysfunction by alloxan (which destroys pancreatic β -cells and induces type 1 diabetes) and a cholesterol-rich diet (which induces obesity and type 2 diabetes) in male Wistar albino rats. Both tea extracts significantly alleviated most signs of the metabolic syndrome including hyperglycaemia (resulting from type 1 and 2 diabetes), dyslipidaemia and impairment of liver functions induced by alloxan or the cholesterol-rich diet in the animals. Also, the tea extracts significantly modulated both the severe decrease and increase in body weight induced by alloxan and the high-cholesterol diet, respectively. The modulatory effects obtained here were partial or complete, but significant and dose dependent, and slightly more in GTE in most cases. No harmful effects were detected for tea consumption on all parameters measured, except that the high dose of both tea extracts significantly decreased the spleen weight:body weight ratio and induced lymphopenia. The present study supports the hypothesis that both black and green teas may have beneficial effects against the risks of the metabolic syndrome and CVD as shown in rat models of human obesity and diabetes.

Albino rats: Alloxan: Hypercholesterolaemia: Hyperglycaemia: Liver dysfunction: Tea

Diabetes, a disorder of metabolism, is increasingly affecting more and more individuals in the world and the number of individuals suffering from diabetes worldwide is predicted to reach 325 million by the year 2025⁽¹⁾. There are two forms of diabetes mellitus: type 1 and 2. In type 1 diabetes or insulin-dependent diabetes mellitus the pancreatic β -cells are progressively destroyed and secrete little or no insulin. Type 2 diabetes or non-insulin-dependent diabetes mellitus is a heterogeneous disorder of insulin resistance and pancreatic β -cell dysfunction^(2,3). About 80% of diabetic morbidity and mortality is caused by cardiomyopathy that is mainly due to diabetic dyslipidaemia⁽³⁾.

Obesity, excess body weight, is a representative lifestyle-related disorder caused by an excessively fat-rich dietary intake associated with a lack of physical exercise⁽⁴⁾. Obesity has increased at an alarming rate in recent years and is now a worldwide health problem, since it is a major risk factor for CVD, type 2 diabetes, impairment of liver functions and even cancer, for which the social costs are incalculable^(5–7).

Hyperglycaemia in addition to alterations in plasma lipids and lipoproteins in streptozotocin-diabetic rats⁽⁸⁾, mice fed a high-fat diet⁽⁹⁾ and obese humans⁽¹⁰⁾ affect cardiovascular circulation and cardiac output and increase the risk of

atherosclerosis and CHD. Now there is a solid body of evidence which links the consumption of tea to a reduced risk of CVD by improving glucose and lipid metabolism.

Tea, from the plant *Camellia sinensis*, is one of the most popular beverages consumed worldwide. Tea is rich in antioxidant polyphenolic flavonoids (catechins, flavonols, theaflavins and thearubigins) that possess various pharmacological effects such as anti-hypertensive, anti-arteriosclerotic, hypoglycaemic and hypocholesterolaemic activity^(8,11–14). Green tea (unfermented tea) is favoured in Asian countries especially Japan and China, whereas black tea (fully fermented tea) is favoured in the Western world⁽²⁾. The processing methods of tea yield preparations with differing chemical compositions and perhaps differing efficacies. During the manufacture of black tea, polyphenol oxidase in fresh tea leaves oxidises catechins (tea tannins) into quinones, which condense to form theaflavins and thearubigins^(2,15,16). This fermentation process is inactivated by steam or pan firing treatment of freshly harvested tea leaves to produce green tea^(2,17). The catechins (epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate) and flavonols (kaempferol, quercetin and myricetin glycosides) of green tea represent 80–90% and < 10% of total flavonoids, respectively. On the other

Abbreviations: A:G, albumin:globulin; ALP, alkaline phosphatase; AMPK, AMP-activated protein kinase; BTE, black tea aqueous extract; GTE, green tea aqueous extract.

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hand, the catechin content of black tea is only 20–30%, whilst the theaflavins and thearubigins represent about 10 and 50–60% of total flavonoids, respectively^(15,16).

Despite the considerable difference in the chemical composition of both tea extracts, studies comparing the glucose- and lipid-lowering activity of black and green tea in rat models of human obesity and diabetes are limited. Therefore, the main purpose of the present study was to compare the modulatory effects of black tea extracts (rich in theaflavins and thearubigins) with green tea extracts (rich in catechins) on experimentally induced hyperglycaemia, hyperlipidaemia and liver dysfunction by alloxan (which destroys pancreatic β -cells) and a cholesterol-rich diet (which induces obesity and insulin resistance) in male Wistar albino rats. Furthermore, the present study investigated any deleterious effects caused by the consumption of black and green tea extracts.

Materials and methods

Chemicals and tea preparations

Alloxan monohydrate ($C_4H_2N_2O_4 \cdot H_2O$; molecular weight 160.08 Da) and cholesterol ($C_{27}H_{46}O$, molecular weight 386.66 Da) were purchased from Sigma-Aldrich (St Louis, MO, USA) and WinLab (Market Harborough, Leics, UK), respectively. Black and green teas (R. Twining & Co. Ltd, London, UK) were purchased from a public market in Cairo, Egypt. Black tea aqueous extract (BTE) and green tea aqueous extract (GTE) were prepared by dissolving amounts equivalent to 50 and 100 mg tea leaf powder per kg body weight in glassware containing 0.5 ml boiling distilled water (equivalent to 1.5 and 3 cups of tea, respectively), then covered and let stand for 10 min at room temperature. After that the extracts were filtered and given fresh to the animals⁽¹⁸⁾.

Experimental animals and treatment schedule

Adult male Wistar albino rats (*Rattus norvegicus*), weighing about 120 g, were obtained from the National Research Centre in Giza, Egypt. Animals were housed in suitable cages and acclimatised to laboratory conditions for a period of 1 week before the commencement of the experiments. Rats were fed standard rodent food pellets (Agricultural-Industrial Integration Company, Giza, Egypt) and double-distilled water. The standard rodent food pellets contain wheat-bran, dried clover, maize, bean-hay, methionine, molasses, salt, in addition to mineral and vitamin mixes. The amount of crude proteins, fats and fibres in the food pellets are 12, 2.4 and 14%, respectively. The energy content of the standard diet is 920.48 kJ/100 g. All animals were humanely treated in accordance with the WHO guideline for animal care and the study design was approved by the Ain Shams University Research Ethics Committee.

Experimental animals were divided randomly into fifteen groups of seven rats each: five healthy groups, five diabetic groups and five obese groups. In healthy groups, animals were treated by oral administration and daily with 50 or 100 mg/kg body weight (low or high dose, respectively) of either BTE or GTE for 4 weeks. The control animals received orally and daily 0.5 ml distilled water, as vehicle, and were housed in cages for 4 weeks under the same conditions as

the other groups. In diabetic groups, animals were injected subcutaneously by alloxan (120 mg/kg body weight) for the first 3 d only to induce hyperglycaemia⁽¹⁹⁾ and given orally and daily the low or high dose of either BTE or GTE for 4 weeks. Alloxan-only-treated animals received orally and daily 0.5 ml distilled water and were housed in cages for 4 weeks under the same conditions as the other groups. In obese groups, animals were fed a cholesterol-rich diet (standard rodent food pellets plus 2% cholesterol, w/w) to induce hyperlipidaemia^(20,21) and given orally and daily the low or high dose of either BTE or GTE for 4 weeks. Cholesterol-only-treated animals received orally and daily 0.5 ml distilled water and were housed in cages for 4 weeks under the same conditions as the other groups.

Blood and tissue sampling

The rats were subjected to light diethyl ether anaesthesia before killing. The blood was collected into clean test-tubes with or without EDTA. A portion of blood with EDTA was used to determine the total and differential leucocyte counts. Another blood portion without EDTA was left to coagulate at room temperature and the clotting time was recorded using a stopwatch. Then, blood was centrifuged in a cooling centrifuge (IEC centra-4R; International Equipment Co., Needham Heights, MA, USA) for 30 min at 3000 rpm and 4°C to separate the clot. The serum was separated at once by a long Pasteur pipette, divided into samples and preserved at -70°C for further analysis. Immediately after killing the animals, the liver and spleen were separated out of the body, cleaned and weighed.

Measurements

Food intake (on a per-group basis) was measured weekly. Body-weight gain or loss was calculated by the following equation: body-weight gain or loss = body weight at the end of the experiment – body weight at the beginning of the experiment. All biochemical analyses were manually done using commercial kits. Serum glucose, total protein, albumin, total lipids, TAG, phospholipids, total cholesterol, HDL-cholesterol and bilirubin (total and direct) concentrations were determined either enzymically or colorimetrically using Diamond Diagnostics (DP International, Cairo, Egypt) and Sentinel CH (Milan, Italy) kits^(22–31). Serum alanine and aspartate aminotransferases and alkaline phosphatase (ALP) activities were estimated either colorimetrically or kinetically using BioMérieux SA (Marcy-l'Etoile, France) and Biocon Diagnostik (Vöhl-Marienhagen, Germany) kits^(32,33).

Serum total globulins and indirect bilirubin concentrations were calculated according to the following equations: total globulins = serum total protein – albumin; indirect bilirubin = total bilirubin – direct bilirubin. LDL-cholesterol concentration was calculated according to the equation of Friedewald *et al.*⁽³⁴⁾: LDL-cholesterol = total cholesterol – (TAG/5) – HDL-cholesterol. Atherogenic indexes were calculated as follows: atherogenic index (1) = total cholesterol:HDL-cholesterol ratio; atherogenic index (2) = LDL-cholesterol:HDL-cholesterol ratio.

Total and differential leucocyte counts were measured by Coulter (Hemat 8 analyser; SEAC, Freiburg, Germany)

using 0.5 ml fresh anti-coagulated blood. The percentage of change of any parameter = $((T - C)/C) \times 100$, where (T) = the mean value of the parameter in the treated group and (C) = the mean value of the parameter in the control group.

Statistics

Data are presented as mean values with their standard errors. Statistical analysis was performed with one-way ANOVA and the differences among groups were determined by Bonferroni's multiple comparison test⁽³⁵⁾ using GraphPad Prism version 4.03 for Windows (GraphPad Software Inc., San Diego, CA, USA). Statistically significant variations were compared as follows: (a) all groups v. the control group; (b) alloxan plus tea extracts-treated groups v. the alloxan-only-treated group; (c) cholesterol plus tea extracts-treated groups v. the cholesterol-only-treated group. *P* values of <0.05 and <0.01 were considered significant and highly significant, respectively.

Results

Modulatory effects of black tea aqueous extract compared with green tea aqueous extract on diabetic rats

The present study showed that treatment of rats with alloxan alone did not significantly alter ($P > 0.05$, $t = 2.597$, difference between means = -0.0024 , 95% CI -0.0053 , 0.0006) the liver weight:body weight ratio, but induced a highly significant increase ($P < 0.01$) in clotting time, serum glucose, total lipid, TAG, phospholipid and bilirubin (total and indirect) levels, total cholesterol:HDL-cholesterol and LDL-cholesterol:HDL-cholesterol ratios, and serum aminotransferase and

ALP activities compared with the control animals (Tables 1–3). On the other hand, it induced a highly significant decrease ($P < 0.01$) in body weight, serum total protein and direct bilirubin levels, and albumin:globulins (A:G) ratio compared with the control animals (Tables 1 and 3).

Both tea extracts significantly alleviated ($P < 0.05$ – $P < 0.01$) the severe decrease in body weight (Table 1) and the increase in serum glucose, total lipid, TAG and phospholipid levels (Tables 1 and 2), atherogenic index values (Table 2), and serum aminotransferase and ALP activities (Table 3) induced by alloxan. These modulatory effects of tea extracts were dose dependent and slightly more in GTE groups compared with BTE groups in most cases. Serum TAG levels became comparable with that of the control group ($P > 0.05$, $t = 3.170$, difference between means = -11.44 , 95% CI -23.28 , 0.40) in the group treated with alloxan plus the high dose of GTE only. There were no significant modulatory effects of both tea extracts on clotting time, serum total protein and bilirubin (total, direct and indirect) levels, and A:G ratio in alloxan plus tea extracts-treated groups compared with the alloxan-only-treated group. Food intake was not significantly changed ($P > 0.05$) in alloxan with or without tea extracts-treated groups compared with the control group (Fig. 1).

The percentages of changes of all parameters measured in the alloxan-only-treated group, groups treated with alloxan plus either the low or high dose of BTE, and groups treated with alloxan plus either the low or high dose of GTE were 124.9 (SEM 54.3), 81.2 (SEM 43.5), 50.9 (SEM 27.5), 79.9 (SEM 47.1) and 40.0 (SEM 22.2), respectively, compared with the control group. All of these results revealed that the most modulatory effects on diabetic rats were induced by the high dose of BTE and GTE.

Table 1. The modulatory effects of tea aqueous extracts on body and liver weight, clotting time, and serum levels of glucose and proteins in healthy, diabetic, and obese rat models

(Mean values with their standard errors)

Group	Body-weight gain or loss (g)		Liver:body weight ratio		Clotting time (s)		Glucose (mg/l)		Total protein (g/l)		A:G ratio	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	45.9	1.1	0.0268	0.0005	56.0	1.6	874	56	46.2	2.4	1.03	0.05
BTE 50	47.9	1.5	0.0262	0.0004	57.0	0.9	791	13	45.8	2.3	1.03	0.06
BTE 100	41.7	1.1	0.0248	0.0002	56.0	1.9	679**	8	46.6	2.0	1.07	0.07
GTE 50	45.3	1.6	0.0267	0.0002	57.3	0.8	774	11	46.6	1.9	1.03	0.05
GTE 100	35.0**	1.9	0.0252	0.0004	57.7	1.3	663**	12	46.0	1.9	0.96	0.05
Alloxan	-15.4**	0.8	0.0292	0.0004	81.1**	5.9	3020**	39	35.3**	2.5	0.64**	0.09
Alloxan + BTE 50	8.9**††	0.8	0.0290	0.0002	77.6**	7.5	1496**††	16	33.8**	1.8	0.40**	0.03
Alloxan + BTE 100	18.1**††	2.5	0.0290	0.0004	79.0**	7.1	1330**††	20	34.5**	1.7	0.86	0.07
Alloxan + GTE 50	16.7**††	2.3	0.0281	0.0007	73.7	5.0	1413**††	16	33.7**	1.3	0.49**	0.03
Alloxan + GTE 100	36.1**††	3.2	0.0274	0.0006	76.7*	5.6	1271**††	13	34.8**	2.0	0.43**	0.06
Cholesterol	97.7**	1.5	0.0427**	0.0009	71.1	0.9	1504**	20	37.9	1.9	0.73**	0.04
Cholesterol + BTE 50	76.9**	1.5	0.0400**	0.0007	69.3	2.5	1217**††	13	37.6	1.3	0.76*	0.07
Cholesterol + BTE 100	55.6**††	3.6	0.0335**††	0.0012	67.0	2.0	1068**††	38	37.7	1.6	0.84	0.05
Cholesterol + GTE 50	70.1**†	1.2	0.0349**††	0.0011	69.4	2.8	1203**††	26	37.7	1.3	0.53**	0.04
Cholesterol + GTE 100	50.3**††	1.7	0.0330**††	0.0006	67.3	2.1	1034**††	27	37.8	1.9	0.59**	0.03

A:G ratio, albumin:globulin ratio; BTE 50, black tea aqueous extract (50 mg/kg body weight); BTE 100, black tea aqueous extract (100 mg/kg body weight); GTE 50, green tea aqueous extract (50 mg/kg body weight); GTE 100, green tea aqueous extract (100 mg/kg body weight).

Mean value was significantly different from that of the control group: * $P < 0.05$, ** $P < 0.01$.

Mean value was significantly different from that of the alloxan-only-treated group: † $P < 0.05$, †† $P < 0.01$.

Mean value was significantly different from that of the cholesterol-only-treated group: ‡ $P < 0.05$, ‡‡ $P < 0.01$.

Table 2. The modulatory effects of tea aqueous extracts on serum lipid profile and atherogenic indexes in healthy, diabetic, and obese rat models (Mean values with their standard errors)

	Total lipids (mg/l)		TAG (mg/l)		Phospholipids (mg/l)		Atherogenic index (1)		Atherogenic index (2)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	4679	167	527	6	1364	14	1.39	0.02	0.14	0.01
BTE 50	4551	186	440	37	1324	7	1.33	0.04	0.11	0.02
BTE 100	3723*	205	318**	9	1304	7	1.23	0.02	0.06	0.01
GTE 50	4260	117	438	29	1320	12	1.32	0.06	0.10	0.03
GTE 100	3464**	226	292**	15	1301	9	1.22	0.02	0.06	0.01
Alloxan	7009**	267	1129**	27	1731**	15	3.34**	0.15	1.44**	0.12
Alloxan + BTE 50	6401**	228	925***††	18	1629***†	27	2.88**	0.13	1.19**	0.10
Alloxan + BTE 100	5927***†	104	660***††	42	1491***††	18	2.15***††	0.15	0.75***††	0.12
Alloxan + GTE 50	6189**	214	764***††	17	1582***††	30	2.89**	0.19	1.31**	0.16
Alloxan + GTE 100	5754***††	281	642***††	23	1487***††	14	2.00***††	0.14	0.63***††	0.11
Cholesterol	8051**	321	1088**	23	2354**	19	4.25**	0.17	2.58**	0.14
Cholesterol + BTE 50	6169***††	171	930***††	32	1587***††	19	2.89***††	0.11	1.25***††	0.10
Cholesterol + BTE 100	5867***††	180	656***††	34	1478***††	17	2.23***††	0.17	0.82***††	0.15
Cholesterol + GTE 50	5939***††	156	853***††	22	1595***††	41	2.88***††	0.07	1.28***††	0.05
Cholesterol + GTE 100	5519***††	126	650***††	21	1466***††	14	2.18***††	0.07	0.76***††	0.05

Atherogenic index (1), total cholesterol:HDL-cholesterol ratio; atherogenic index (2), LDL-cholesterol:HDL-cholesterol ratio; BTE 50, black tea aqueous extract (50 mg/kg body weight); BTE 100, black tea aqueous extract (100 mg/kg body weight); GTE 50, green tea aqueous extract (50 mg/kg body weight); GTE 100, green tea aqueous extract (100 mg/kg body weight).

Mean value was significantly different from that of the control group: * $P < 0.05$, ** $P < 0.01$.

Mean value was significantly different from that of the alloxan-only-treated group: † $P < 0.05$, †† $P < 0.01$.

Mean value was significantly different from that of the cholesterol-only-treated group: ‡ $P < 0.01$.

Modulatory effects of black tea aqueous extract compared with green tea aqueous extract on obese rats

The present study showed that feeding rats a cholesterol-rich diet did not significantly alter ($P > 0.05$) the clotting time and serum total protein level (Table 1), but induced a significant increase ($P < 0.05$ – $P < 0.01$) in body weight, liver weight:

body weight ratio, serum glucose, total lipid, TAG, phospholipid and bilirubin (total and indirect) levels, total cholesterol:HDL-cholesterol and LDL-cholesterol:HDL-cholesterol ratios, and serum aminotransferase and ALP activities compared with the control animals (Tables 1–3). On the other hand, it induced a highly significant decrease in A:G

Table 3. The modulatory effects of tea aqueous extracts on serum aminotransferases and alkaline phosphatase activities and bilirubin level in healthy, diabetic, and obese rat models

(Mean values with their standard errors)

	ALAT activity (IU/l)		ASAT activity (IU/l)		ALP activity (IU/l)		Bilirubin (mg/l)					
							Total		Direct		Indirect	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	30.6	1.1	40.7	1.0	29.8	1.1	14.11	0.24	12.34	0.13	1.77	0.29
BTE 50	28.4	1.8	38.1	0.8	28.6	0.8	14.31	0.07	12.40	0.19	1.91	0.16
BTE 100	23.9*	0.4	35.6	1.4	24.3	0.7	14.86	0.18	12.80	0.19	2.06	0.29
GTE 50	27.9	0.8	37.8	1.5	26.7	0.9	14.44	0.26	12.43	0.15	2.01	0.28
GTE 100	23.5*	0.6	35.1	0.7	22.9*	0.9	14.87	0.13	12.87	0.11	2.00	0.10
Alloxan	89.9**	0.9	148.7**	1.8	99.9**	3.0	15.09**	0.21	9.81**	0.29	5.27**	0.28
Alloxan + BTE 50	63.2***††	2.0	82.1***††	1.4	52.4***††	1.8	14.94*	0.15	8.50**	0.36	6.44**	0.44
Alloxan + BTE 100	39.7***††	0.4	67.6***††	2.4	40.0***††	0.8	14.91*	0.12	8.54**	0.39	6.37**	0.47
Alloxan + GTE 50	61.4***††	1.9	79.5***††	0.9	47.5***††	1.0	14.89	0.11	9.41**	0.59	5.47**	0.61
Alloxan + GTE 100	39.0***††	1.1	61.2***††	2.0	38.4***††	0.7	14.86	0.12	9.50**	0.24	5.36**	0.29
Cholesterol	90.8**	2.2	129.2**	1.3	45.5**	1.4	14.97*	0.18	9.81**	0.18	5.16**	0.21
Cholesterol + BTE 50	54.8***††	1.9	60.3***††	2.2	38.3***†	1.6	14.96*	0.16	9.46**	0.39	5.50**	0.37
Cholesterol + BTE 100	41.3***††	1.5	48.7***††	1.1	36.8***††	0.8	14.96*	0.10	9.69**	0.22	5.27**	0.20
Cholesterol + GTE 50	46.5***††	1.2	54.2***††	0.8	38.0***††	1.4	14.90	0.07	9.56**	0.26	5.34**	0.23
Cholesterol + GTE 100	39.7***††	1.3	48.4***††	1.2	35.7***††	1.5	14.89	0.17	9.63**	0.35	5.26**	0.33

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; ALP, alkaline phosphatase; BTE 50, black tea aqueous extract (50 mg/kg body weight); BTE 100, black tea aqueous extract (100 mg/kg body weight); GTE 50, green tea aqueous extract (50 mg/kg body weight); GTE 100, green tea aqueous extract (100 mg/kg body weight).

Mean value was significantly different from that of the control group: * $P < 0.05$, ** $P < 0.01$.

Mean value was significantly different from that of the alloxan-only-treated group: †† $P < 0.01$.

Mean value was significantly different from that of the cholesterol-only-treated group: ‡ $P < 0.05$, ‡‡ $P < 0.01$.

ratio ($P < 0.01$, $t = 3.788$, difference between means = 0.30, 95 % CI 0.04, 0.55) and serum direct bilirubin level ($P < 0.01$, $t = 6.027$, difference between means = 0.25, 95 % CI 0.12, 0.39) compared with the control animals (Tables 1 and 3).

Both tea extracts significantly alleviated ($P < 0.05$ – $P < 0.01$) the increase in body weight and liver weight:body weight ratio (Table 1), serum glucose, total lipid, TAG and phospholipid levels (Tables 1 and 2), atherogenic index values (Table 2), and serum aminotransferase and ALP activities (Table 3) induced by the cholesterol-rich diet. These modulatory effects of tea extracts were dose dependent and slightly more in the GTE groups compared with the BTE groups in most cases. Body-weight gain, serum total lipid and TAG levels, and serum ALP activity became comparable with those of the control group ($P > 0.05$) in the group that received the cholesterol-rich diet plus the high dose of GTE only. There were no significant modulatory effects of both tea extracts on A:G ratio and serum bilirubin (total, direct and indirect) level in groups that received the cholesterol-rich diet plus tea extracts compared with the group that received the cholesterol-rich diet only. Food intake was not significantly altered ($P > 0.05$) in cholesterol-rich diet with or without tea extracts-treated groups compared with the control group (Fig. 1).

The percentages of changes of all parameters measured in the group that received the cholesterol-rich diet only, groups that received the cholesterol-rich diet plus either the low or high dose of BTE, and groups that received the cholesterol-rich diet plus either the low or high dose of GTE were 170.9 (SEM 94.4), 83.9 (SEM 43.7), 50.9 (SEM 27.9), 78.0 (SEM 45.2) and 44.9 (SEM 26.1), respectively, compared with the control group. All of these data indicated that the most modulatory effects on obese rats were induced by the high dose of BTE and GTE.

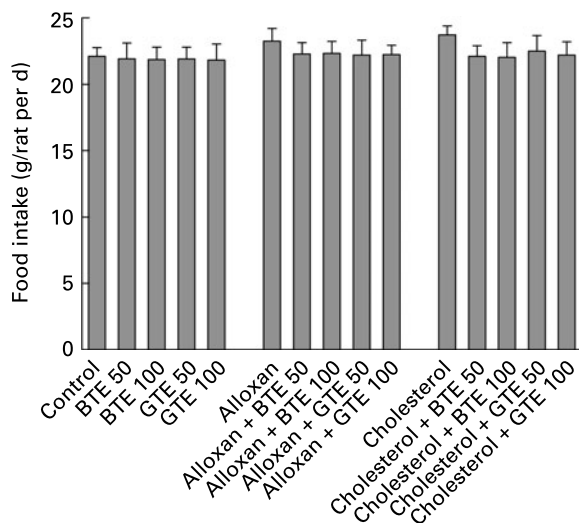


Fig. 1. Food intake (g/rat per d) in healthy, diabetic, and obese rat models treated with or without tea extracts. BTE 50, black tea aqueous extract (50 mg/kg body weight); BTE 100, black tea aqueous extract (100 mg/kg body weight); GTE 50, green tea aqueous extract (50 mg/kg body weight); GTE 100, green tea aqueous extract (100 mg/kg body weight). Values are means, with standard errors represented by vertical bars.

Beneficial and deleterious effects caused by black tea aqueous extract and green tea aqueous extract consumption in healthy rats

The present study showed that treatment of rats with tea extracts alone did not significantly affect ($P > 0.05$) the liver weight:body weight and A:G ratios, clotting time, serum total protein, phospholipid and bilirubin (total, direct and indirect) levels, atherogenic index values, and serum aspartate aminotransferase activity compared with the control animals (Tables 1–3). Food intake was not significantly affected ($P > 0.05$) in the tea extracts-treated groups compared with the control group (Fig. 1). Also, tea consumption did not significantly alter ($P > 0.05$) the globulin fractions (α_1 , α_2 , β and γ globulins), kidney functions (serum urea and creatinine levels), erythrocyte and platelet counts, Hb content, packed cell volume value and blood indices in healthy rats (data not shown). On the other hand, rats treated with the high dose of either BTE or GTE showed a significant decrease ($P < 0.05$ – $P < 0.01$) in serum glucose, total lipid and TAG levels (Tables 1 and 2), and alanine aminotransferase activity (Table 3) compared with the control animals. In addition, only rats treated with the high dose of GTE showed a significant decrease in body-weight gain ($P < 0.01$, $t = 3.998$, difference between means = 10.86, 95 % CI 1.95, 19.77) and serum ALP activity ($P < 0.05$, $t = 3.594$, difference between means = 6.94, 95 % CI 0.60, 13.27) compared with the control animals. All of these observations indicated that the most beneficial effects on healthy rats were induced by the high dose of BTE and GTE.

The only deleterious effects of tea consumption detected in the present study were the significant reduction in spleen weight:body weight ratio ($P < 0.05$) and the induction of leucopenia ($P < 0.01$), which is mainly due to lymphopenia ($P < 0.01$), in rats that received the high dose of either BTE or GTE only compared with the control animals (Figs. 2 and 3).

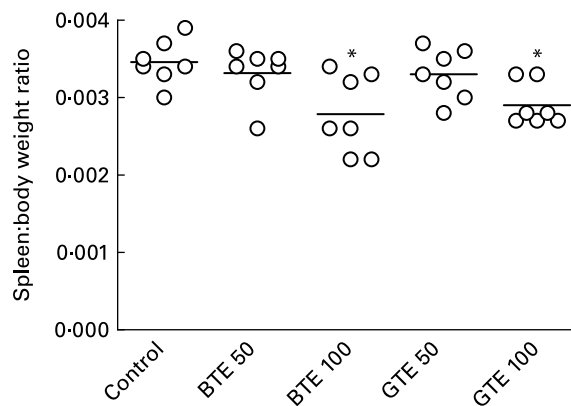


Fig. 2. Spleen weight:body weight ratio in control and tea-only-treated animals. BTE 50, black tea aqueous extract (50 mg/kg body weight); BTE 100, black tea aqueous extract (100 mg/kg body weight); GTE 50, green tea aqueous extract (50 mg/kg body weight); GTE 100, green tea aqueous extract (100 mg/kg body weight). Individual values are shown, with means represented by horizontal bars. *Mean value was significantly different from that of the control group ($P < 0.05$).

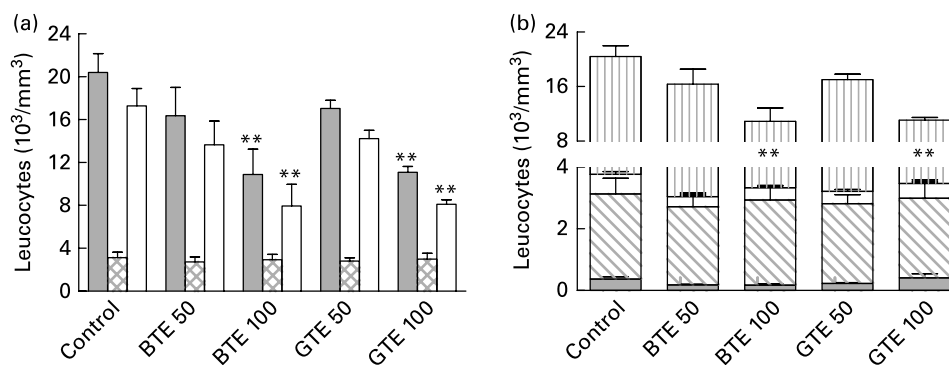


Fig. 3. (a) Total leucocyte counts in control and tea-only-treated animals. (□), Total leucocytes; (XX), total granulocytes; (□), total agranulocytes. (b) Differential leucocyte counts in control and tea-only-treated animals. (XX), Basophils; (■), eosinophils; (▨), neutrophils; (□), monocytes; (▩), lymphocytes. BTE 50, black tea aqueous extract (50 mg/kg body weight); BTE 100, black tea aqueous extract (100 mg/kg body weight); GTE 50, green tea aqueous extract (50 mg/kg body weight); GTE 100, green tea aqueous extract (100 mg/kg body weight). Values are means, with standard errors represented by vertical bars. ** Mean value was significantly different from that of the control group ($P < 0.01$).

Discussion

Alloxan produces oxygen radicals, which destroy pancreatic β -cells and cause severe hypoinsulinaemia (type 1 diabetes) that is responsible for the hyperglycaemia and dyslipidaemia seen in alloxan-treated animals^(17,36). However, its action is not directed to pancreatic β -cells only, as other organs such as the liver, kidney and bone marrow are also affected by alloxan administration as seen from the elevation of plasma markers reflecting hepatic and renal cell damage (alanine aminotransferase activity, total:direct bilirubin ratio, and urea and creatinine levels) and the reduction of haematological parameters⁽¹⁷⁾. Weight loss (one of the clinical features of type 1 diabetes) seen in alloxan-treated animals may be due to the degeneration of the adipocytes and muscle tissues to compensate for the energy lost from the body due to frequent urination and over-conversion of glycogen to glucose⁽³⁷⁾. On the other hand, there is a strong positive correlation between a high-cholesterol diet and increased production of reactive oxygen species and decreased activities of endogenous radical scavengers that lead to increased incidence of the metabolic syndrome, a clustering of pathological conditions including obesity, dyslipidaemia, hepatic steatosis and insulin resistance (type 2 diabetes)^(7,38). Cardiovascular complications are a major cause of morbidity and mortality in patients with diabetes, obesity and the metabolic syndrome^(39–41).

The present study showed that both tea extracts significantly alleviated most signs of the metabolic syndrome including hyperglycaemia (resulting from type 1 and 2 diabetes), hyperlipidaemia and impairment of liver functions induced by alloxan or a cholesterol-rich diet in male albino rats. The hypolipidaemic effect and the marked decrease in atherogenic indexes shown in the present study by tea extracts in alloxan or high-cholesterol diet-treated rats (Table 2) may reduce the incidence of atherosclerosis in diabetic and obese patients. The most interesting finding in the present study was that tea extracts significantly modulated both the severe decrease and increase in body weight induced by alloxan and the high-cholesterol diet, respectively (Table 1). Maintenance of a constant body weight requires a balance between cellular energy intake and energy expenditure. A slight imbalance in this energy equilibrium can lead to significant changes in

body weight and may eventually result in obesity or slimness⁽⁶⁾. In the present study, neither daily nor total food intake was significantly changed during the treatment period among all groups (Fig. 1). The significant increase in body-weight gain induced by tea extracts in the diabetic rats, despite similar food consumption, suggests that BTE and GTE may have a positive anabolic effect by decreasing the degeneration of the adipocytes and muscle tissues through improving glucose metabolism. On the other hand, the significant decrease in body-weight gain and liver weight:body weight ratio induced by both BTE and GTE in the obese rats may be due to a reduction in body and liver fats by improving lipid metabolism, increasing energy expenditure, and affecting fat absorption and excretion. Thus, the results of the present study suggest a direct effect of tea extracts on the improvement of metabolism rather than affecting food intake.

Several other studies reported that the hypoglycaemic effect of tea was attributed to the presence of polyphenols, catechins and a water-soluble polysaccharide fraction^(17,42–45). Also, it was found that tea consumption significantly decreased blood glucose levels by increasing hepatic glycogen level in alloxan-diabetic rats possibly through reactivation of the glycogen synthase system (as a result of increased insulin secretion) and decreasing liver glucose-6-phosphatase activity, which is mainly responsible for releasing glucose molecules to the blood by converting glucose-6-phosphate to glucose^(17,36,46). Moreover, Anandh Babu *et al.*⁽⁸⁾ reported that the increase in insulin-stimulated glucose uptake, inhibition of the intestinal GLUT system and decrease in expression of genes that control gluconeogenesis are the mechanisms proposed to be responsible for the anti-hyperglycaemic effect of tea.

BTE has been found to quench reactive oxygen species such as singlet oxygen, superoxide and hydroxyl radicals⁽¹⁸⁾, which may explain its hypoglycaemic activity obtained in the present study. Other studies reported that green tea epigallocatechin gallate promotes pancreatic β -cell regeneration in alloxan-treated rats, has insulin-like and insulinotropic activities, and inhibits gluconeogenesis through inhibition of liver phosphoenolpyruvate kinase synthesis^(3,46,47). The study of Wolfram *et al.*⁽⁴⁸⁾ also demonstrated that green tea epigallocatechin gallate possesses pronounced anti-diabetic efficacy in preclinical

models of type 2 diabetes mellitus, which is at least partially mediated through reducing the hepatic glucose production and enhancing the pancreatic function.

Based on biochemical and pharmacological studies, the mechanisms of preventing obesity by tea consumption may be through stimulating hepatic lipid metabolism, inhibiting gastric and pancreatic lipases, stimulating thermogenesis and suppressing fatty acid synthase by black tea theaflavins and green tea catechins^(4,6). Both black and green tea leaves have been found to decrease plasma TAG, cholesterol and LDL-cholesterol in rats by decreasing lipogenesis⁽⁴⁹⁾. In addition, black and green tea polyphenols were found to modulate the hypercholesterolaemia in the experimental animals by up-regulating the liver LDL receptor, inhibiting cholesterol synthesis, and increasing faecal excretion of cholesterol, total fatty acids and bile acid^(50–52).

BTE supplementation has the ability to restore the activity of antioxidant enzymes, reduce the generation of free radicals, blunt lipid peroxidation and prevent hepatocellular damage in rats receiving ethanol with or without a high-fat diet⁽¹⁸⁾. Moreover, black tea theaflavins has the ability to reduce hepatic lipid accumulation both *in vitro* and *in vivo*⁽⁵³⁾. The release of liver alanine aminotransferase, aspartate aminotransferase and ALP was inhibited in rats probably by chemical components of black tea that may stabilise the integrity of the cell membrane and keeping the membrane intact and the enzymes enclosed through scavenging free radicals⁽⁵⁴⁾. Thus, the hepatoprotective effect of BTE found in the present study may be due to inhibition of the oxidative stress, induced by alloxan and the cholesterol-rich diet, by increasing cellular antioxidant capacity and reducing membrane lipid peroxidation. On the other hand, it has been found that green tea epigallocatechin gallate inhibited obesity, the metabolic syndrome, hepatic steatosis and the release of liver enzymes induced by a high-fat diet in mice⁽⁷⁾. Other studies reported that green tea epigallocatechin gallate inhibited lipid absorption through its ability to form complexes with lipids and lipolytic enzymes and hence interfering with the luminal processes of emulsification, hydrolysis, micellar solubilisation, and subsequent uptake of lipids^(55,56). Green tea epicatechins and epigallocatechin gallate have been found to modulate the increase in LDL-cholesterol, probably by decreasing the apoB (the principal protein that comprises nearly 90% of total protein mass of LDL), and the decrease in HDL-cholesterol in experimental animals fed a high-fat or high-cholesterol diet^(57,58).

Flavonols are less affected by tea processing and are present in comparable amounts in both green and black teas^(59,60). Theaflavins present in black tea possess the same antioxidant potency as catechins present in green tea, and the conversion of catechins to theaflavins during fermentation in making black tea does not alter significantly their free radical-scavenging activity⁽⁶¹⁾. In the past few years, it has been reported that AMP-activated protein kinase (AMPK) plays a key role in regulating carbohydrate and fat metabolism, serving as a metabolic master switch in response to alterations in cellular energy charge⁽⁵³⁾. AMPK is physiologically activated by the phosphorylation of threonine 172 within the α -subunit catalysed by the kinase LKB1, the upstream kinase of AMPK⁽⁶²⁾. AMPK activation results in the phosphorylation and inhibition of acetyl-CoA carboxylase, in addition to the

loss of inhibition of carnitine palmitoyl transferase I by decreasing the concentration of malonyl-CoA, leading to increased fatty acid oxidation. AMPK also regulates glucose homeostasis by modulating gluconeogenesis-related molecules, such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in the liver. In addition, AMPK activation inhibits adipocyte differentiation and suppresses the expression of lipogenic molecules, such as fatty acid synthase, acetyl-CoA carboxylase and PPAR- γ ⁽⁶³⁾. Therefore, based on the central role of AMPK in the regulation of energy metabolism, it may be a promising molecular target for the suppression of obesity and the treatment of the metabolic syndrome. Recently, it was found that both black tea theaflavins and green tea catechins significantly attenuated the high glucose-induced insulin signalling blockade, reduced lipid accumulation, suppressed fatty acid synthesis, and stimulated fatty acid oxidation through activating the LKB1-AMPK pathway in cultured cells and BALB/c mice^(53,63,64). All of these findings may rationalise the striking similarity in the functional effects of BTE and GTE obtained in the present study, despite the considerable difference in the chemical composition of both tea extracts.

All modulatory effects obtained in the present study were increased by increasing the dose of tea extracts. The modulatory effects of the highest dose of GTE were slightly more in most cases in the present study. In addition, only the high dose of GTE modulated completely hypertriglyceridaemia induced by alloxan and the cholesterol-rich diet. Also, the significant elevations in body weight, serum total lipid level, and serum ALP activity induced by the cholesterol-rich diet were completely modulated by the high dose of GTE only. Although the difference in effect size between the highest dose of GTE and BTE was very subtle for each parameter measured here, the modulatory effects for all parameters together in the obese and diabetic rats were from 6.0 (SEM 2.6) to 10.9 (SEM 6.3) % higher in the groups that received the high dose of GTE than in those that received the high dose of BTE. This may be because green tea contains more antioxidant compounds compared with black tea as reported by Steele *et al.*⁽⁶⁵⁾.

No modulatory effects were detected for both tea extracts on A:G ratio and serum bilirubin levels in both animal models used in the present study. This may be due to the doses used here, which can be considered low. Another interesting finding of the present study was that no harmful effects were detected for tea consumption on all parameters measured, except that the high dose of both tea extracts significantly decreased spleen weight:body weight ratio (Fig. 2), probably due to decreasing its cellularity, and induced lymphopenia (Fig. 3). This may be beneficial in alleviating the undesirable hyperimmune response found in autoimmune diseases such as rheumatoid arthritis. This topic needs further investigation and will be considered in the future.

In general, the modulatory effects of BTE and GTE on some parameters measured in the present study were partial, but significant, and dose dependent. Therefore, it may be more beneficial if the dose increased in future studies. The present study supports the hypothesis that both black and green teas may have beneficial effects against the risks of the metabolic syndrome and CVD as shown in rat models of human obesity and diabetes.

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N. M. E.-B. and G. R. planned the study, designed all experiments, summarised, discussed and interpreted the results, and drafted the manuscript. E. A. A. E.-G. carried out the experiments and performed the statistical analysis with assistance from G. R.

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