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Comparative Studies on the Hypolipidemic and Growth Suppressive Effects of Oolong, Black, Pu-erh, and Green Tea Leaves in Rats

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The four major commercial teas, oolong, black, pu-erh, and green teas, have been manufactured in southeast Asia. In this study, we evaluated the growth suppressive and hypolipidemic effect of these four different tea leaves by oral feeding to male Sprague−Dawley rats for 30 weeks. The results showed that the suppression of body weights of tea leaves-fed groups were in the order: oolong tea > pu-erh tea > black tea > green tea. Pu-erh tea and oolong tea could lower the levels of triglyceride more significantly than that of green tea and black tea, but pu-erh tea and green tea were more efficient than oolong tea and black tea in lowering the level of total cholesterol. In lipoprotein, 4% pu-erh tea could increase the level of HDL-C and decrease the level of LDL-C, but other teas simply decrease the levels of both. The activity of antioxidant enzyme SOD is increased in all tea-fed groups as compared to the basal diet-fed group. Finally, relative weight ratios of liver to epididylmal adipose tissue were lower in feeding oolong tea and pu-erh tea groups. On the basis of these findings, it seemed that the fully fermented pu-erh and black tea leaves and partially fermented oolong tea leaves were more effective on their growth suppressive and hypolipidemic effects as compared to the nonfermented green tea leaves.

KEYWORDS: Gowth suppressive; hypolipodemic; triglyceride; total cholesterol; HDL-C; LDL-C

INTRODUCTION

It is generally observed that obese people are seldom found in the long-term tea drinking individuals group. In 1998, a significant hypolipidemic and growth suppressive effect of green tea was observed in rats after 63 weeks of feeding (*1*). Recently, a significant suppression of fatty acid synthase (FAS) by tea and tea polyphenols has been demonstrated in the human breast carcinoma MCF-7 cells and hepatocellular carcinoma Hep-G2 cells (*2*). These interesting findings have promoted us to investigate the hypolipidemic and growth suppressive effects of oolong, black, pu-erh, and green teas in Sprague−Dawley rats. The purpose of this study is try to answer the simple question that green tea has been reported to show hypolipidemic and anti-obesity effects in experimental animals (*1*), but how about the effects of other teas, oolong, black, and pu-erh teas, under similar conditions? The definitive answer to this question will provide very important information to the tea-drinkers when they are purchasing tea leaves for health promotion.

Tea plants are widely cultivated in China, Sri Lanka, Indonesia, Japan, India, Taiwan, and central African countries. Tea has an attractive aroma, taste, and healthy effects, so it has become one of the most popular beverages in the world. To base them on the degree of fermentation, generally, they are classified into three major categories: the nonfermented green tea, the partially fermented oolong or paochong tea, and the fully fermented black or pu-erh tea (*3*).

The composition of tea varies with species, season, age of the leaf (plucking position), climate, and horticultural practices (*4*). Tea contains polyphenols, especially green tea, which include flavanols, flavadiols, flavonoids (*5*), and phenolic acids that may account for up to 30% of the dry weight. Certain catechins are the most biologically active group of the polyphenols in tea components. The major tea catechins are (−)-epigallocatechin 3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin 3-gallate (ECG), (−)-epicatechin (EC), (+)-gallocatechin (GC), and (+)-catechin (C). During the fermentation process, polyphenol oxidase in tea oxidized catechin into quinone and then condensed to form bisflavanol, theaflavin, thearubigen, and other high molecular components (*6*).

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Much evidence indicates that tea polyphenols have various biological activities including anti-fungal (*7*), anti-inflammation (*8*), anti-mutagenic (*9*), anti-oxidative (*10*), anti-carcinogenic (*11*), antitumor (*12*) effects, lowering of plasma cholesterol and triglyceride levels, and reduction of blood pressure and platelet aggregation in several systems (*13*, *14*).

Hypercholesterolemia is a major risk factor in atherosclerosis and coronary heart disease, while maintaining the blood cholesterol level within the normal range can reduce the risk of having these pathological changes. Current drug treatments of hypercholesterolemia involve the use of statin, fibrates, and bile acid binding resins. They lower the cholesterol level by decreasing de novo synthesis of cholesterol through inhibition of HMG-CoA reductase, affecting lipoproteins metabolism and reducing absorption of cholesterol, respectively. It has been shown that tea could lower the plasma cholesterol (*6*, *14*). Catechins, the most abundant components in green tea, have been implicated for its hypocholesterolemic effect. The level of blood triglyceride is another important risk factor in the pathogenesis of atherosclerosis and coronary heart diseases. It is very encouraging to find that the long-term feeding of green tea powder to rats could reduce the blood level of triglyceride and other lipids (*1*). In the present study, we have investigated the effects of the tea leaves with different degrees of fermentation on lipid levels and antioxidant in male SD rats by oral feeding.

MATERIALS AND METHODS

Chemicals. Reagent kits for total cholesterol, triglyceride, LDL-C, HDL-C, and SOD were purchased from Randox (Randox Laboratories, Antrim, UK). Green tea leaves, oolong tea leaves, and black tea leaves were manufactured by Wun-Shan Branch, Tea Research and Extension Station (Taipei, Taiwan). The fresh tea leaves were plucked from tea plant TTES#12 which is a well-established species developed by The Taiwan Tea Experiment Station and were stored at 4 °C in a sealed bag. Pu-erh tea leaves were purchased from the tea commercial market in Taipei. The pu-erh tea was imported from Kuang-Shi, China. In the experiment, all tea leaves were crushed into powder and thoroughly mixed with basal diet.

Animals and Treatment. Male Sprague−Dawley (SD) rats (5 weeks old) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The rats were housed in stainless steel wire-bottomed cages and acclimated under laboratory conditions (19−23 °C, humidity 60%, 12-h light/dark cycle) for at least 1 week before each study. The weights of rats at the beginning of the study ranged from 150 to 200 g. All rats were weighed every week. Free access to ground Purina rat chow (Ralston Purina, St. Louis, MO) and water was permitted prior to the experimental period. After 1 week of acclimation, the rats were fed different diets: 1.5% and 4% green tea leaves, 1.5% and 4% oolong tea leaves, 1.5% and 4% black tea leaves, 1.5% and 4% pu-erh tea leaves, and basal diet. The experiment was terminated after 30 weeks. The rat were then ether-anesthetized, blood was collected from the jugular vein, serum was separated for the estimation of total cholesterol, triglyceride, HDL-C, and LDL-C, and liver and epididymal adipose tissue were weighed and frozen at −70 °C.

Triglyceride Assay. This was done by the GPO-PAP method (Randox Laboratories, Antrim, U.K.). Triglycerides are enzymatically hydrolyzed to glycerol and free fatty acids by special lipases. In the subsequent enzymatic oxidation by glycerol kinase and glycerol phosphatase, H_2O_2 is formed. This is converted into a colored quinonimine in a reaction with 4-aminoantipyrine and phenol catalyzed by peroxidase, which was determined spectrophotometrically at 546 nm. The unit of the content of triglyceride was expressed as milligrams per deciliter.

Cholesterol Assay. This was estimated by the CHOD-PAP method (Randox Laboratories, Antrim, U.K.). Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyzes the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H_2O_2 is formed. This is converted into a colored quinonimine in a reaction with 4-aminoantipyrine and phenol catalyzed by peroxidase, which was determined spectrophotometrically at 546 nm. The unit of the content of cholesterol was expressed as milligrams per deciliter.

HDL-Cholesterol Assay. Low-density lipoproteins (LDL and VLDL) are specifically precipitated by phosphotungstic acid and magnesium ions and can then be removed by centrifugation. HDL remains in the supernatant (Randox Laboratories, Antrim, U.K.). Determination of HDL-C is performed using the clear supernatant. This is estimated by the CHOD-PAP method. The unit of the content of HDL-C was expressed as milligrams per deciliter.

LDL-Cholesterol Assay. LDL is precipitated by heparin at their isoelectric point (pH 5.12). After centrifugation, the HDL and the VLDL remain in the supernatant and can then be determined by enzymatic methods (Randox Laboratories, Antrim, UK). LDL-C = total cholesterol − cholesterol in the supernatant. The unit of the content of LDL-C was expressed as milligrams per deciliter.

Assay of Superoxide Dismutase Activity in Serum. Four hundred microliters of ice-cold absolute ethanol/chloroform 62.5:37.5 (v/v) was added to 250 μL of serum in a glass test tube and then thoroughly mixed for at least 30 s and centrifuged at 3000*g* for 5 min at 4 °C. The resulting supernatant was stored between 2 and 8 °C until used for the assay. Serum SOD activity was determined by the commercial kit from Randox (Randox Laboratories).

Statistical Analysis of the Data. The results obtained were expressed as the mean ± SE, and the significance of the difference (p value) was statistically analyzed by ANOVA followed by Dunnett test's (*15*) to assess the statistical significance ($p < 0.05$) between tested and control groups through all experiments.

RESULTS

Effect of Tea Leaves on the Body Weight and Dietary Intake of Rats. The body weights of rats were examined when 5-week-old male SD rats were fed the basal diet, 1.5%, and 4% green, oolong, black, and pu-erh tea leaves. The weights of rats in each group after 30 weeks are given in **Figure 1**. At the 30 weeks, feeding 1.5% green tea leaves had produced no reduction in the body weight, but 1.5% oolong tea ($p < 0.01$), black tea ($p < 0.05$), and pu-erh tea ($p < 0.005$) had remarkably decreased the body weight as compared to the basal diet-fed group. In the feeding of 4% tea leaves, the rats were all remarkably reduced in body weight. At 30 weeks, green tea-fed was about 6% ($p < 0.005$), oolong tea-fed was 11% ($p < 0.001$), black tea-fed was about 7% ($p < 0.001$), and pu-erh tea-fed was about 13% ($p < 0.005$) lower than basal diet-fed. The dietary intakes of SD rats in each group are shown in **Table 1** and show no significant differences throughout the feeding period. The average body weights of rats in each group at 0, 5, 15, and 30 weeks of feeding are illustrated in **Table 2**.

Levels of Catechins in the Tested Tea Leaves. Table 3 shows the concentration of catechins in water extract of the four tea leaves as analyzed by HPLC method (*3*). Much higher levels of catechins were observed in green tea and oolong tea. The major catechins present were epigallocatechin (EGC), epigallocatechin gallate (EGCG), and epicatechin (EC). The levels of those catechins in black tea and pu-erh tea were significantly lower than those in green and oolong tea.

Effect of Tea Leaves on Serum Triglycerides. Figure 2 illustrates serum triglycerides levels of SD rats fed different tea leaves for 30 weeks. In the green tea group, the concentration of serum triglycerides in the 1.5% and 4% tea leaves-fed groups had a slight increase as compared to the basal diet group at the 15th week, and a slight decrease at the 30th week, but it did not make a significant difference (**Figure 2A**). In the oolong tea group, feeding 1.5% tea leaves by the 15th week caused a significant increase in the triglyceride level ($p < 0.05$), but no

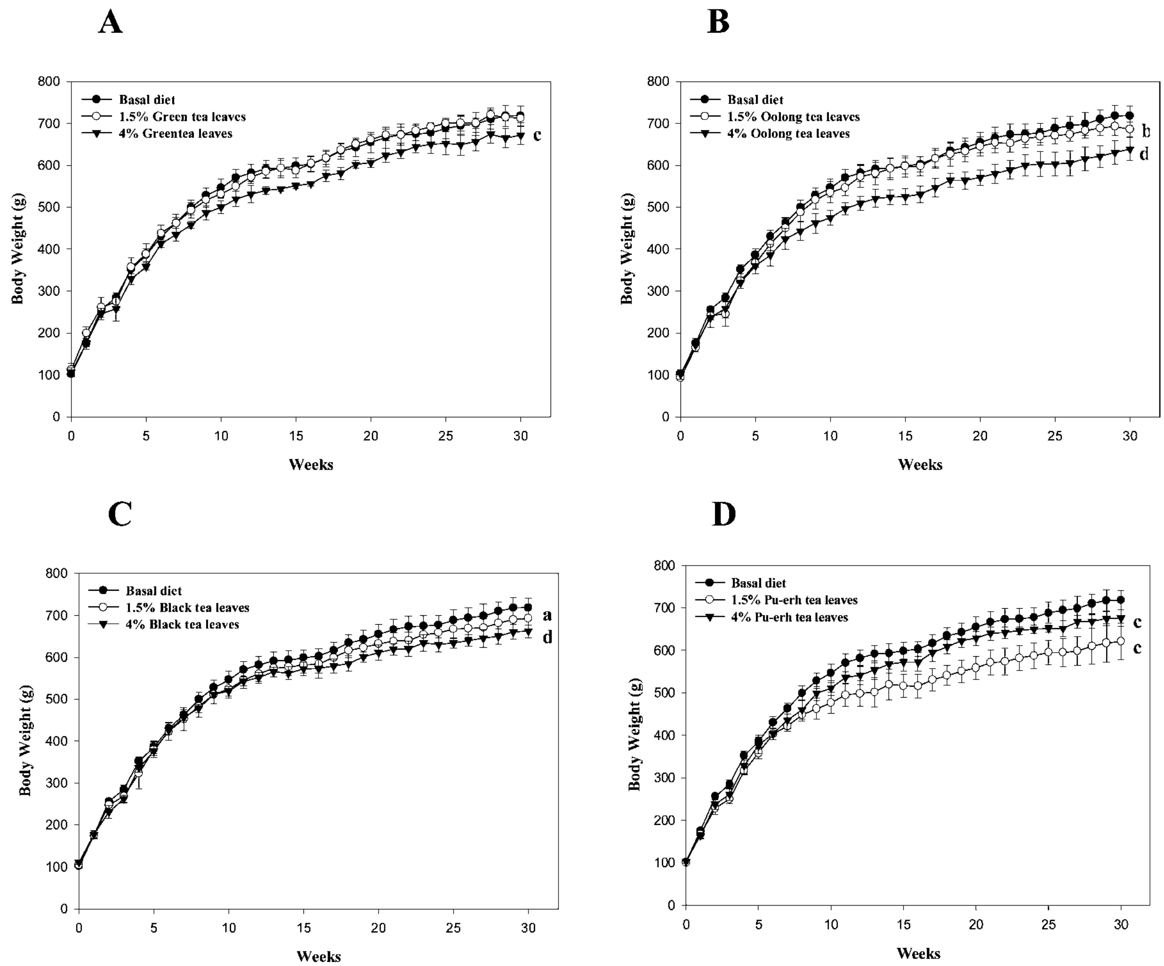

Figure 1. Effects of green, oolong, black, and pu-erh tea leaves on the growth of SD rats. The effects of green tea leaves (**A**), oolong tea leaves (**B**), black tea leaves (**C**), and pu-erh tea leaves (**D**) on the growth of SD rat were measured by the procedure described in the Materials and Methods. Data are presented as the mean ± SE from 10 rats per group. Statistically different from the control group: (a) $p < 0.05$; (b) $p < 0.01$; (c) $p < 0.005$; (d) $p < 0.001$.

Table 1. Dietary Intake (Grams per Day per Rat) of SD Rats Fed on 1.5% and 4% Tea Leaves

	week after initiation of treat ment feeding period (week)									
group[a]	2	4	6	8	10	15	20	25	30	mean ± SE[b]
control (basal diet)	19.0	25.9	31.9	31.9	30.0	26.6	29.2	28.4	27.7	28.62 ± 3.6
1.5% green tea leaves	19.7	26.9	31.5	32.2	29.3	27.4	30.6	30.0	26.4	29.24 ± 3.3
4% green tea leaves	18.0	25.8	36.9	36.8	34.4	31.7	31.1	25.2	27.3	32.48 ± 5.9
1.5% oolong tea leaves	18.5	26.5	33.3	33.5	31.6	29.2	31.5	31.4	29.0	30.56 ± 4.3
4% oolong tea leaves	18.5	26.3	37.0	37.6	34.8	33.5	29.9	27.3	25.0	32.78 ± 5.5
1.5% black tea leaves	18.7	25.2	31.0	32.1	29.3	27.3	29.1	28.3	26.7	28.59 ± 3.6
4% black tea leaves	18.8	27.4	33.1	33.1	30.7	29.7	30.5	28.7	28.2	30.04 ± 4.0
1.5% pu-erh tea leaves	20.6	27.5	37.1	38.3	34.5	29.1	29.9	29.4	28.0	32.85 ± 4.9
4% pu-erh tea leaves	16.7	26.1	32.4	35.8	32.9	31.5	28.9	28.9	27.6	30.97 ± 5.4

[a] The number of rats in each group is 10. [b] $p > 0.1$, no significant difference.

significant decrease at 30 weeks of feeding. In the 4% tea leaves feeding, about a 40% decrease was observed after 30 weeks (**Figure 2B**). The level of serum triglyceride of the 1.5% black tea-fed group slightly decreased at 15 weeks and was significantly lower than that of the basal diet-fed after 30 weeks of feeding ($p < 0.05$). Yet the 4% black tea-fed group was not significantly different (**Figure 2C**). 1.5% pu-erh tea decreased the level of serum triglyceride after 30 weeks of feeding ($p < 0.005$), and the 4% pu-erh tea leaves-fed group was clearly decreased as compared to the basal diet-fed group at the 15th and 30th weeks ($p < 0.05$ and $p < 0.0005$, respectively).

Effect of Tea Leaves on Serum Total Cholesterol. The results in **Figure 3** show the levels of total cholesterol in the serum of SD rats fed 1.5% and 4% tea leaves for 30 weeks. In the green tea leaves group, feeding 1.5% tea leave for 15 weeks caused a slight increase but remarkably decreased by about 24% at 30 weeks ($p < 0.0005$). In 4% green tea-fed, the total cholesterol increased at 15 weeks ($p < 0.01$) but decreased about 20% at 30 weeks ($p < 0.001$) (**Figure 3A**). In feeding with oolong tea leaves (**Figure 3B**), the 1.5% tea leaves-fed group slightly increased the level of serum total cholesterol at the 15th week and slightly decreased at the 30th week. Total cholesterol

Table 2. Average Body Weights of Rats in Each Group at 0, 5, 15, and 30 Weeks of Feeding[a]

	average body weight (g) (mean ± SE, 10 rats/group) feeding period (week)			
group	0	5	15	30
control (basal diet)	101.6 ± 4.8	351.7 ± 10.7	598.6 ± 19.2	718.1 ± 23.4
1.5% green tea leaves	112.0 ± 15.7	356.8 ± 23.3	587.8 ± 17.5	712.7 ± 10.8
4% green tea leaves	106.6 ± 10.4	328.8 ± 13.4[c]	551.4 ± 7.0[c]	671.4 ± 21.4[b]
1.5% oolong tea leaves	93.0 ± 3.5	324.0 ± 17.7[c]	597.1 ± 25.2	685.3 ± 17.3[b]
4% oolong tea leaves	101.4 ± 11.7	319.8 ± 13.9[c]	524.8 ± 19.6[c]	638.0 ± 27.2[c]
1.5% black tea leaves	104.6 ± 7.1	323.4 ± 19.3[c]	581.6 ± 19.7	692.6 ± 26.0
4% black tea leaves	111.1 ± 5.2	336.4 ± 11.7[b]	572.0 ± 16.0[b]	662.1 ± 15.8[c]
1.5% pu-erh tea leaves	100.2 ± 4.7	315.6 ± 8.0[c]	516.2 ± 26.9[c]	620.8 ± 43.7[c]
4% pu-erh tea leaves	103.0 ± 3.2	327.6 ± 17.6[c]	571.6 ± 21.5	675.8 ± 19.2[b]

[a] Data represent mean ± SE ($n = 10$). For statistical significance, Dunnett test's was used between basal diet-fed control and another tea leaves-fed experimental group. [b] Statistically significant versus control; $p < 0.01$. [c] Statistically significant versus control; $p < 0.001$.

Table 3. Polyphenol Composition of Various Fresh Tea Leaves in Water Extract[a]

	mg/g of tea leaves						
	caffeine	EGC	EC	C	EGCG	GCG	ECG
green tea leaves	3.34 ± 0.40	33.33 ± 2.48	1.24 ± 0.06	0.15 ± 0.03	8.95 ± 0.98	0.14 ± 0.01	0.84 ± 0.13
oolong tea leaves	3.35 ± 0.25	2.36 ± 0.81	1.16 ± 0.05	0.49 ± 0.20	8.68 ± 0.22	0.13 ± 0.03	0.83 ± 0.02
black tea leaves	4.08 ± 0.84	1.74 ± 0.29	0.27 ± 0.04	1.46 ± 0.24	0.17 ± 0.13	0.46 ± 0.05	ND[b]
pu-erh tea leaves	3.64 ± 0.34	2.67 ± 0.54	0.11 ± 0.02	0.25 ± 0.10	0.15 ± 0.07	ND	ND

[a] Each of the tea leaves (1 g) was extracted with 100 mL of boiling water, 30 min. Each value represents mean ± SE of three individual determinations. [b] Not detectable.

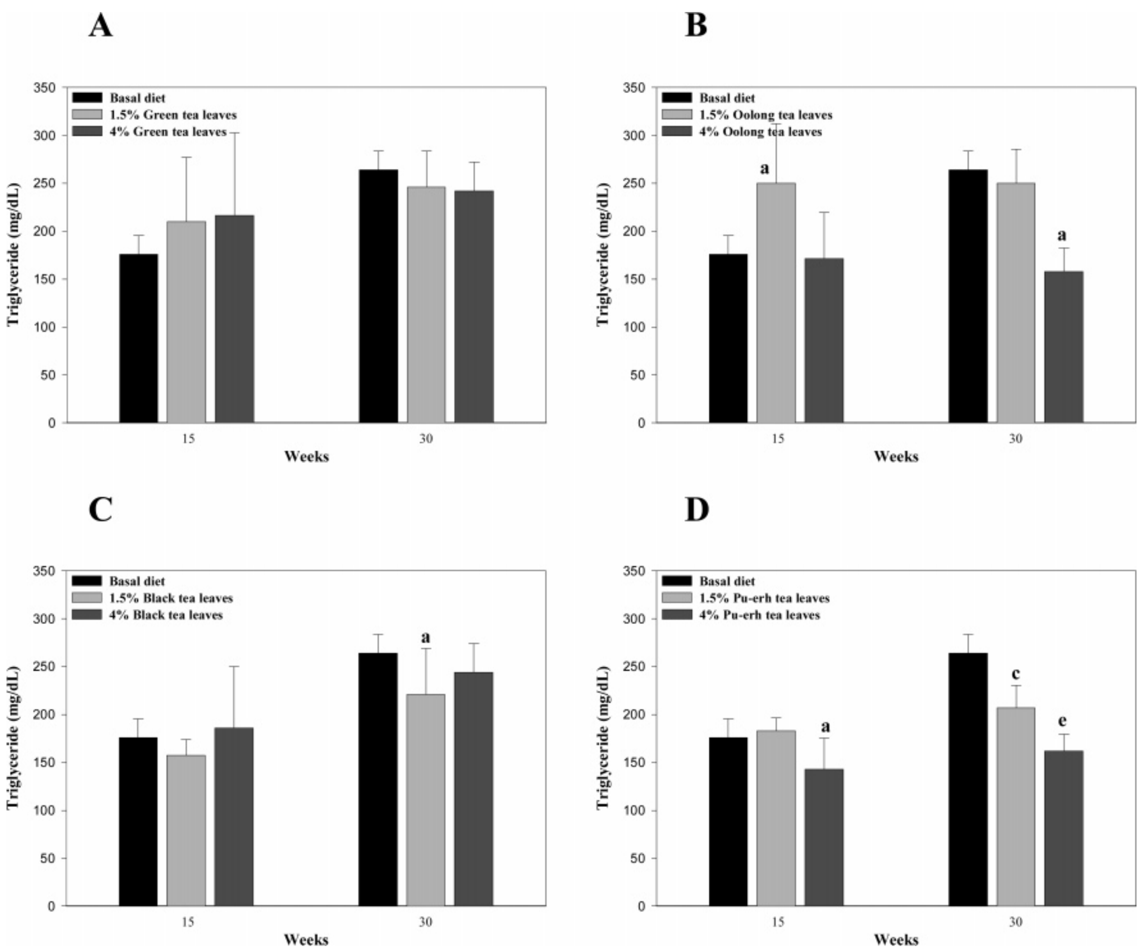

Figure 2. Effects of green, oolong, black, and pu-erh tea leaves on the serum triglyceride level in SD rats. The effects of green tea leaves (**A**), oolong tea leaves (**B**), black tea leaves (**C**), and pu-erh tea leaves (**D**) on the serum triglyceride levels in SD rats were estimated by the method described in the Materials and Methods. Data are presented as the mean ± SE from 10 rats per group. Statistically different from the control group: (a) $p < 0.05$; (b) $p < 0.01$; (c) $p < 0.005$; (d) $p < 0.001$.

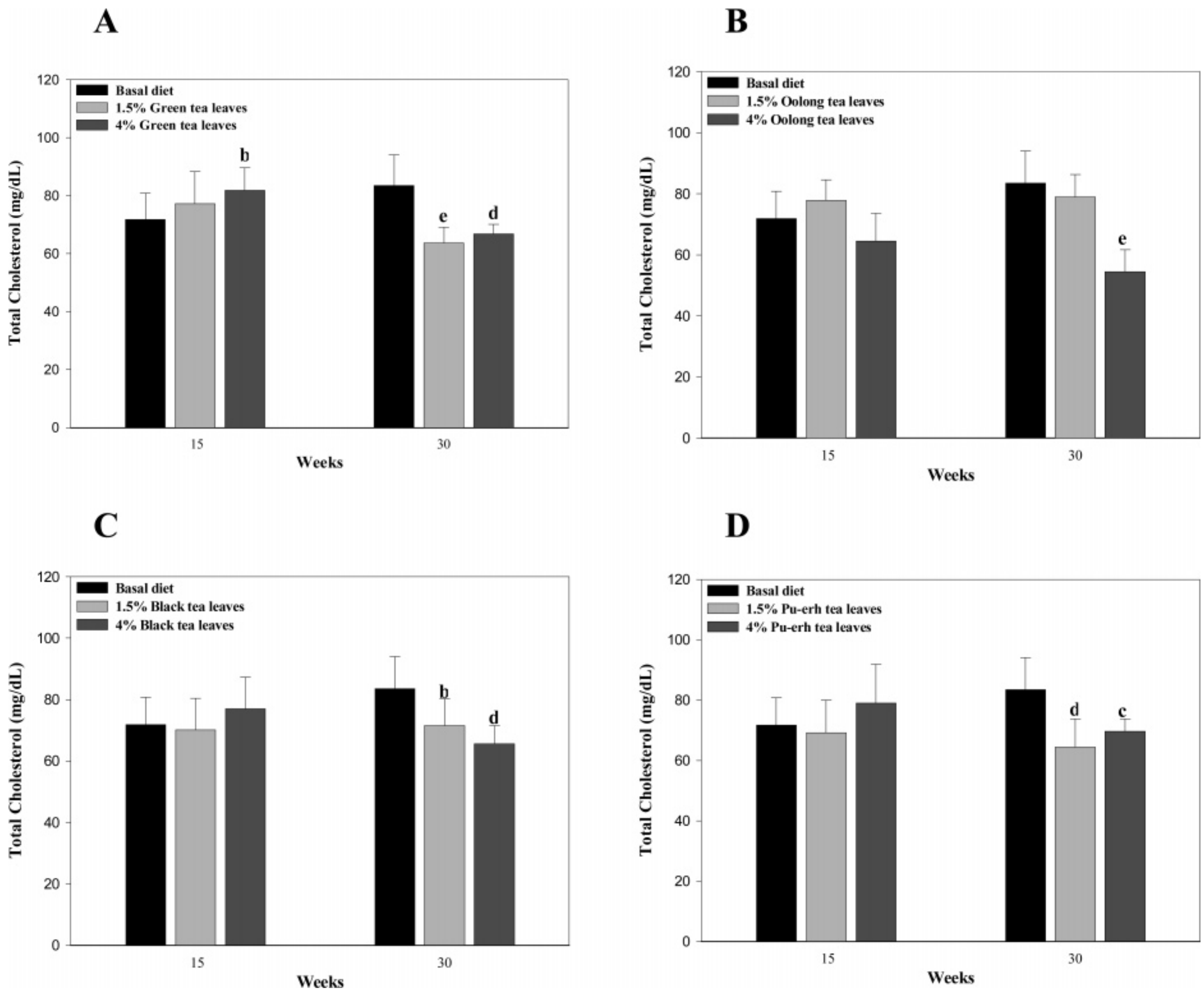

Figure 3. Effects of green, oolong, black, and pu-erh tea leaves on serum total cholesterol in SD rats. The effects of green tea leaves (**A**), oolong tea leaves (**B**), black tea leaves (**C**), and pu-erh tea leaves (**D**) on the serum total cholesterol levels in SD rats were estimated by the method described in the Materials and Methods. Data are presented as the mean ± SE from 10 rats per group. Statistically different from the control group: (a) $p < 0.05$; (b) $p < 0.01$; (c) $p < 0.005$; (d) $p < 0.001$; (e) $p < 0.0001$.

level in the 4% tea leaves-fed group was slightly decreased at the 15th week and dramatically decreased by about 35% at the 30th week ($p < 0.005$). In the 1.5% black tea leaves-fed group, the total cholesterol was slightly decreased at the 15th week and about 15% decreased at the 30th week. In feeding 4% black tea leaves, total cholesterol in serum was slightly increased at the 15th week but decreased about 22% at the 30th week ($p < 0.001$) (**Figure 3C**). Feeding 1.5% pu-erh tea leaves caused no significant decrease at the 15th week but an about 23% decrease at the 30th week. The 4% pu-erh tea leaves group was slightly increased and did not have any significant difference at the 15th week, but was about 17% lower than the basal diet-fed group (**Figure 3D**).

Effect of Tea Leaves on Serum HDL-Cholesterol. Figure 4 shows the effect on HDL-cholesterol after feeding four different kinds of tea leaves after 30 weeks. 1.5% green tea decreased HDL-C at 30 weeks, but feeding 4% green tea caused no significant difference as compared to the basal diet group (**Figure 4A**). The 4% oolong tea decreased the HDL-C level at 15 and 30 weeks ($p < 0.01$). Although the level of serum HDL-C in 1.5% black tea decreased at 15 and 30 weeks, it did not have a significant difference as compared to the basal diet-fed group. In the 1.5% black tea group, we observed the level of HDL-C slightly decrease at 15 and 30 weeks, and 4% black tea-fed also decreased at 30 weeks ($p < 0.05$) (**Figure 4C**). Feeding 4% fully fermented pu-erh tea increased the serum HDL-C at the 15th and 30th week ($p < 0.05$), but 1.5% pu-erh tea caused no significant difference as compared to the basal diet-fed group (**Figure 4D**).

Effect of Tea Leaves on Serum LDL-Cholesterol. The level of serum LDL-C in 1.5% green tea was slightly increased at the 15th week but remarkably about 44% lower than the basal diet group. The same phenomenon was found in the 4% green tea group, which increased the serum LDL-C level at 15 weeks ($p < 0.01$) but clearly about 50% lowered the levels of serum LDL-C as compared to the basal diet group (**Figure 5A**). 1.5% oolong tea also increased the LDL-C level ($p < 0.05$) at the 15th week but decreased about 15% LDL-C at the 30th week. Feeding 4% oolong tea decreased the level of serum LDL-C about 25% and 40% at the 15th and 30th weeks, respectively (**Figure 5B**). The 1.5% and 4% black tea-fed groups, which were not remarkably different from the basal diet group in serum LDL-C at the 15th week, were about 30% and 34% lower in serum LDL-C as compared to the basal diet group at the 30th week (**Figure 5C**). The pu-erh tea leaves-fed group had a slightly decreased LDL-C in the 1.5% and 4% groups at the 15th week and was clearly about 44% and 43% decreased at 30 weeks in feeding 1.5% and 4% tea leaves, respectively (**Figure 5D**).

Effect of Tea Leaves on the Activity of Superoxide Dismutase in Serum. The results of the tea leaves effect on the activity of superoxide dismutase (SOD) in serum are shown

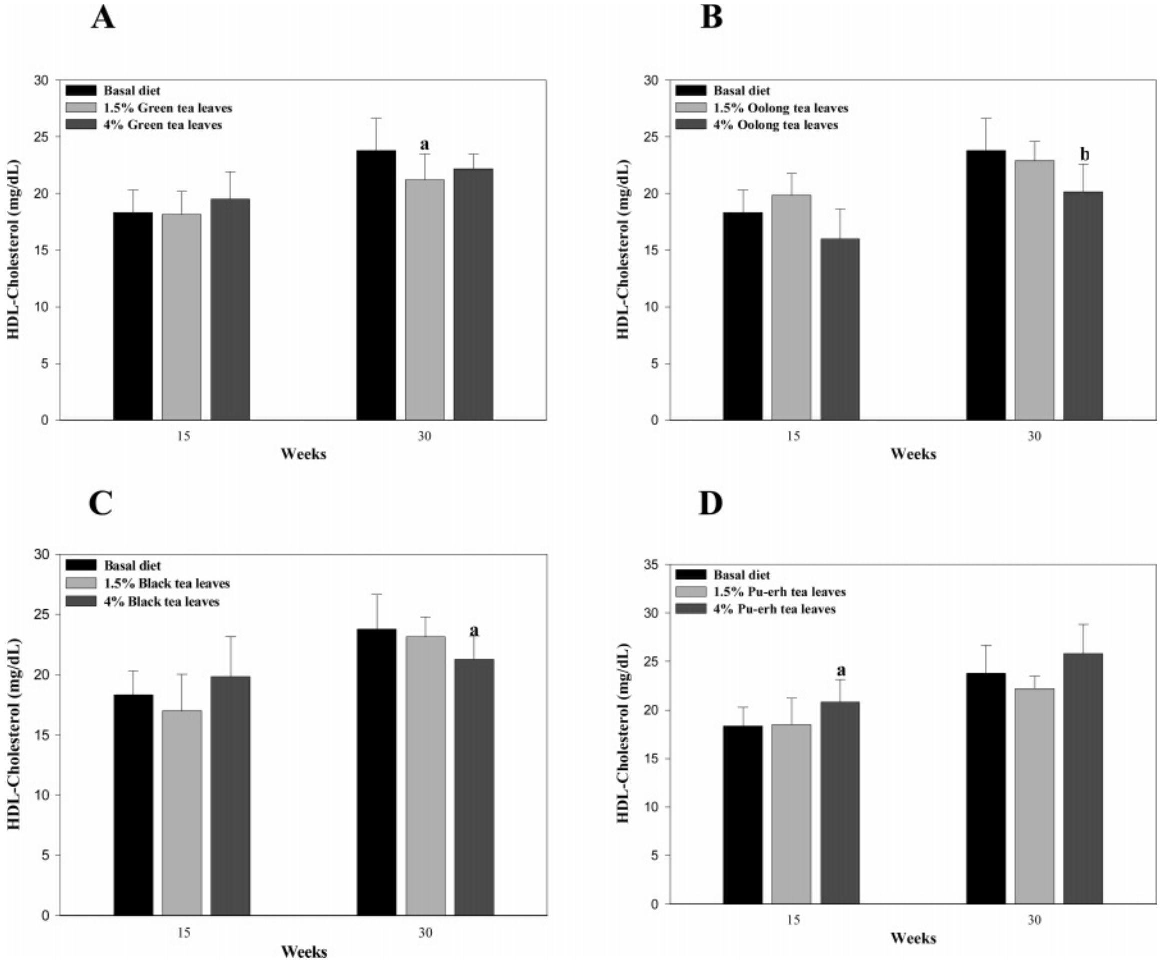

Figure 4. Effects of green, oolong, black, and pu-erh tea leaves on serum HDL-cholesterol in SD rats. The effects of green tea leaves (**A**), oolong tea leaves (**B**), black tea leaves (**C**), and pu-erh tea leaves (**D**) on the serum HDL-cholesterol in SD rats were estimated by the method as described in the Materials and Methods. Data are presented as the mean ± SE from 10 rats per group. Statistically different from the control group: (a) $p < 0.05$; (b) $p < 0.01$; (c) $p < 0.005$; (d) $p < 0.001$.

in **Figure 6**. Feeding 1.5% green tea leaves slightly increased the SOD activity at the 15th and 30th weeks, but it was not significantly different from the basal diet-fed group. In the 4% green tea leaves-fed group, the activity of SOD increased at the 15th and 30th weeks ($p < 0.05$). This effect also was observed in the group of 4% oolong tea leaves ($p < 0.05$). Yet the group of 1.5% oolong tea leaves decreased the SOD activity after 30 weeks of feeding ($p < 0.005$). The activity of SOD in the 1.5% and 4% black tea leaves group also increased after 30 weeks of feeding ($p < 0.005$ and $p < 0.001$, respectively), but it did not make a difference after 15 weeks of feeding (**Figure 6C**). In the pu-erh tea group, it showed no difference at 1.5% and 4% tea leaves feeding as compared to the basal diet-fed group after 30 weeks. Yet at 15 weeks, feeding 4% pu-erh tea leaves decreased the SOD activity ($p < 0.05$).

Effects of Tea Leaves on Liver and Adipose Tissue Weights. The weights of liver and epididymal adipose tissue were determined. The relative liver weight was lower in all tea leaves-fed groups, but feeding with 4% green tea leaves and 1.5% oolong tea leaves at 30 weeks was not statistically significant (**Figure 7A**). In the relative weight of epididymal adipose tissue, feeding 1.5% and 4% oolong tea leaves ($p < 0.05$ and $p < 0.005$, respectively) and 4% pu-erh tea leaves ($p < 0.005$) led to a significant decrease, but other groups showed no significant difference (**Figure 7B**). Based on these data, it is found that the ratios of liver weight to epididymal adipose tissue were significantly lower in the feeding oolong tea and pu-erh tea groups (**Figure 7A**,**B**).

DISCUSSION

The important role of plasma cholesterol and lipoprotein concentration in atherosclerosis and coronary heart disease has been reported by many scientists. High levels of plasma cholesterol, LDL-C, and VLDL-C lead to a high risk for the development of coronary heart disease (*16*) and atherosclerosis (*17*). The association of the serum lipids with coronary heart disease has been demonstrated in middle-aged men and, to a lesser extent, in middle-aged women (*18*−*21*). The coronary heart disease risk is positively associated with total cholesterol and LDL-C and inversely associated with HDL-C in middle-aged populations. Cigarette smoking, diabetes mellitus, and above-optimal blood pressure are also important coronary heart disease risk factors. Recently, our laboratory had demonstrated that oral feeding 2.5% green tea leaves to Wistar rat for 63 weeks could lower the level of serum triglyceride, total cholesterol, and LDL-C (*1*). These results strongly suggested that green tea leaves exerted a hypolipidemic effect and therefore might have a protective effect against the atherosclerotic process. In this study, besides the green tea, feeding the partially fermented oolong tea leaves and fully fermented black and pu-erh tea leaves for 30 weeks also have reduced plasma cholesterol and lipoprotein concentration.

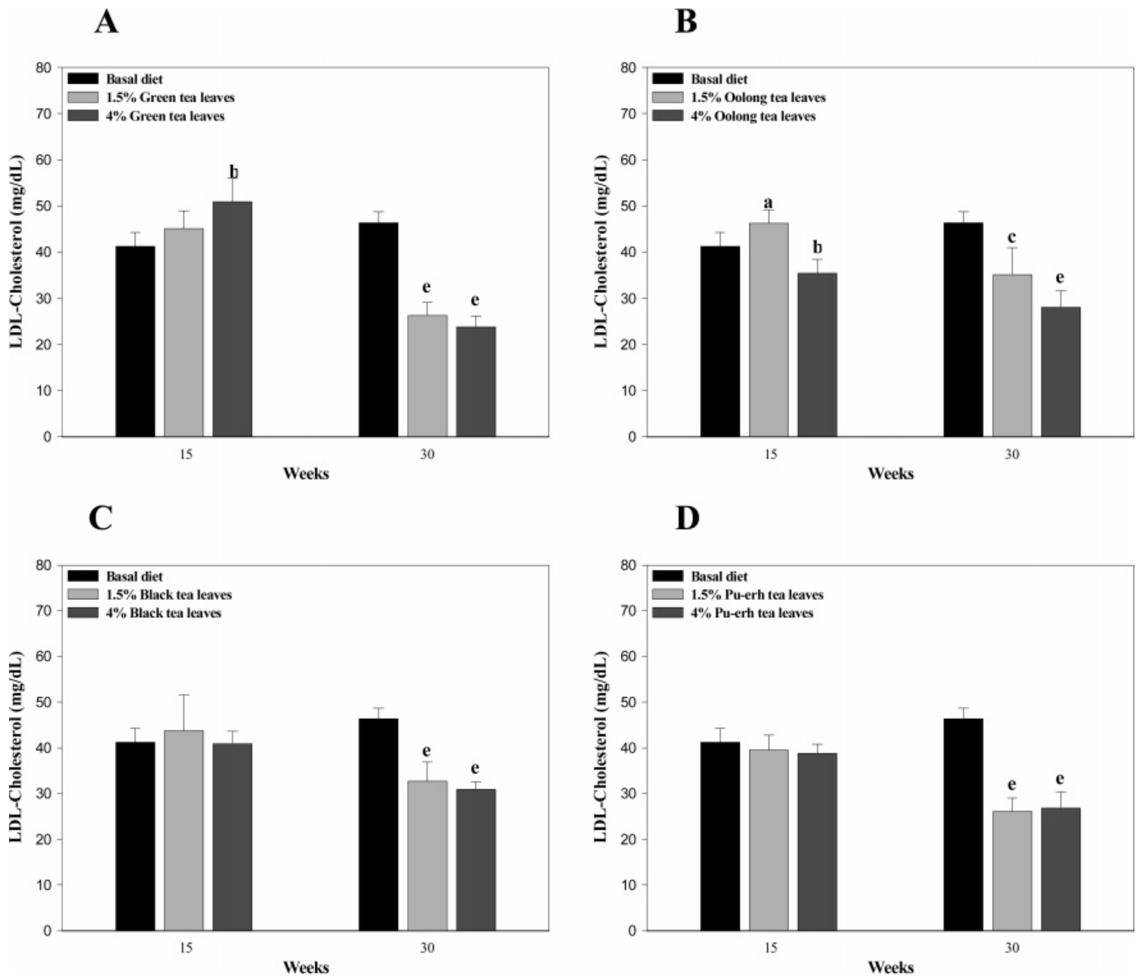

Figure 5. Effects of green, oolong, black, and pu-erh tea leaves on serum LDL-cholesterol level in SD rats. The effects of green tea leaves (**A**), oolong tea leaves (**B**), black tea leaves (**C**), and pu-erh tea leaves (**D**) on the serum LDL-cholesterol levels in SD rats were estimated as described in the Materials and Methods. Data are presented as the mean ± SE from 10 rats per group. Statistically different from the control group: (a) $p < 0.05$; (b) $p < 0.01$; (c) $p < 0.005$; (d) $p < 0.001$; (e) $p < 0.0001$.

In **Table 1** and **Figure 1**, we found that the dietary intakes of the basal diet-fed and tea leaves-fed group were approximately the same, but the body weight of the green tea leaves-fed group was about 6%, oolong-tea leaves-fed group was about 4−11%, black tea leaves-fed group was about 3−7%, and pu-erh tea leaves-fed group was 5−13% lower than those of the basal diet-fed group. Feeding partially fermented oolong tea leaves and fully fermented pu-erh tea leaves seems to have a stronger effect in lowering body weight than nonfermented green tea leaves. As demonstrated in our previous study on green tea (*1*), the extent of reduction in body weight may be increased if the feeding period is extended to 63 weeks or longer. The time-dependent reduction in body weight can be seen in **Table 2**. In the serum triglyceride, **Figure 2** indicated that the fully fermented pu-erh tea leaves-fed and partially fermented oolong tea leaves-fed groups showed stronger lowering effects on the levels of serum triglyceride than the nonfermented green tea leaves-fed group. It seemed to have the same lowering effect between nonfermented green tea leaves, fully fermented pu-erh tea leaves, and black tea leaves in the level of total cholesterol, but partially fermented 4% oolong tea leaves had the most strong effect. Only the fully fermented pu-erh tea group significantly increased the level of lipoprotein HDL-C. Green tea, black tea, and oolong tea groups were decreased at the 30th week. Regarding another lipoprotein level, LDL-C, all tea leaves-fed groups had clearly lower amounts than the basal diet-fed group, and the lower LDL-C was in the order: pu-erh tea > green tea > oolong tea > black tea. For the effects of lipoprotein, we suggest that certain compounds which are derived from tea catechins and are synthesized during the fermentative process of pu-erh tea could affect the level of lipoproteins.

LDL oxidation plays an important role in the development of atherosclerosis (*22*). Oxidized LDL inhibited the macrophage motility, promoted atherosclerosis, and was uptaken by macrophage scavenger receptor, resulting in the stimulation of cholesterol accumulation and hence foam cell formation. Recently, Luo et al. (*23*) reported the inhibition of LDL oxidation by green tea extract, and Mangiapane et al. (*24*) indicated that (+)-catechin inhibited the Cu^{2+}-catalyzed oxidation of human LDL in a dose-dependent manner with complete inhibition at 20 μg/mL. Although the mechanism of inhibition of Cu^{2+}-mediated LDL oxidation by tea polyphenols is unclear now, they might reduce the formation of free radical, or protect α-tocopherol and other antioxidants in LDL, maintaining their levels longer and delaying the start of lipid oxidation (*25*, *26*).

Reactive oxygen species might be important causative agents for a number of human diseases, including cancer, atherosclerosis, and aging. Previously, we have demonstrated that EGCG was a more potent scavenged peroxyl radical than other tea polyphenols (*4*). In this study, we found that the SOD activity in the serum was increased in the tea leaves-fed groups. The

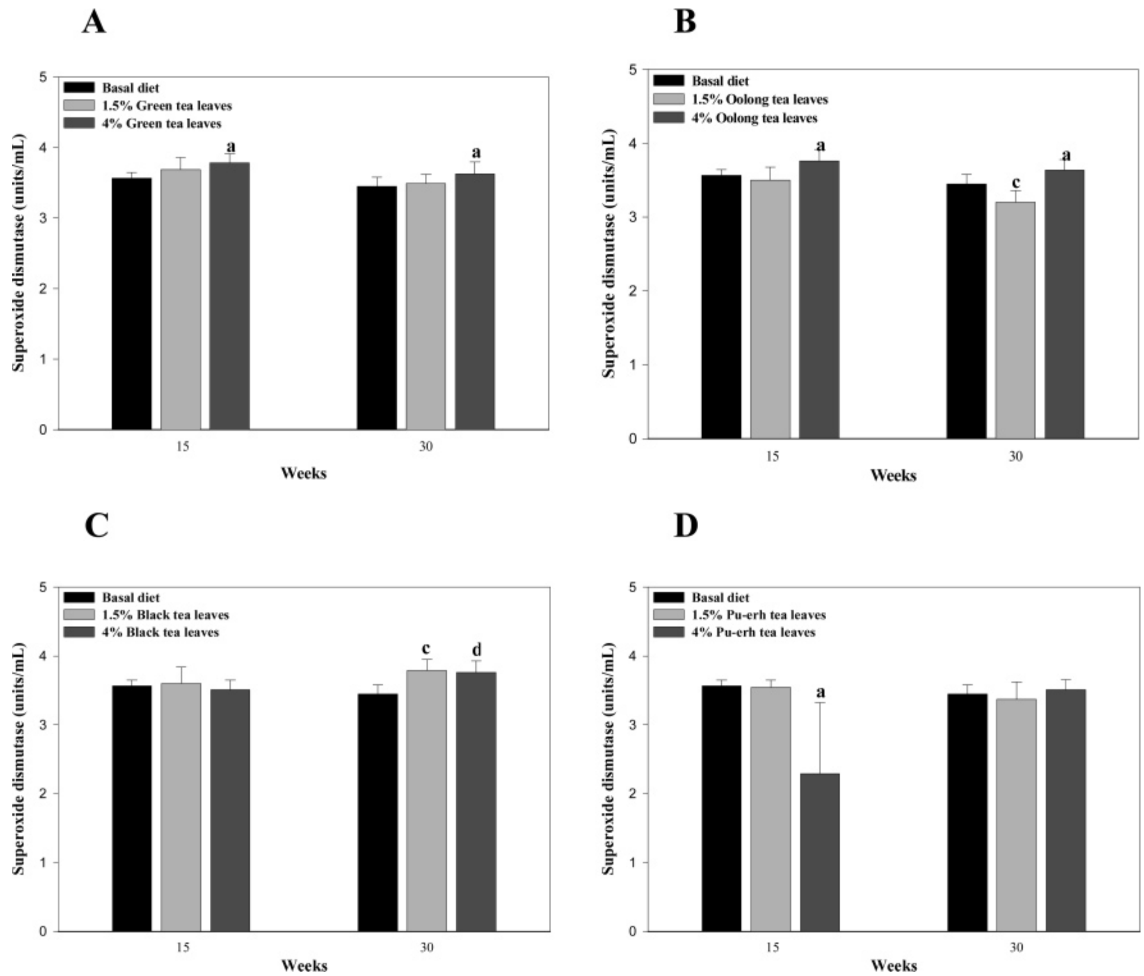

Figure 6. Effects of green, oolong, black, and pu-erh tea leaves on the activity of superoxide dismutase in the serum of SD rats. The effects of green tea leaves (**A**), oolong tea leaves (**B**), black tea leaves (**C**), and pu-erh tea leaves (**D**) on the activity of superoxide dismutase in the serum of SD rats were estimated as described in the Materials and Methods. Data are presented as the mean ± SE from 10 rats per group. Statistically different from the control group: (a) $p < 0.05$; (b) $p < 0.01$; (c) $p < 0.005$; (d) $p < 0.001$.

SOD can then remove the superoxide anion radicals which can produce cytotoxicity and genotoxicity. Yet the order of increased serum SOD was black tea > oolong tea > green tea > pu-erh tea. We suggest that some unidentified new polyphenols may play the same role with EGCG during the processes of fermentation and their ROS scavenging activity may be even stronger than EGCG in vivo.

Fatty acid synthase (FAS) is an important enzyme participating in energy metabolism (*27*) and is related to various human diseases including obesity, cardiovascular disease, and cancer. FAS inhibitor clearly activates potent weight-reducing pathways, so compounds that regulate convergent hypothalamic pathways and/or novel neuropeptide systems activated by FAS inhibitor might be promising candidates for anti-obesity drugs. Recently, our group has demonstrated that black tea polyphenol theaflavins could strongly inhibit FAS activity through the PI-3K/AKT/Sp-1 pathway (*2*). In **Figure 7**, we observed the relative weight of epididymal adipose tissue decrease in 1.5% and 4% oolong tea leaves-fed and 4% pu-erh tea leave groups. We suggested that there may be certain compounds in fully fermented pu-erh tea which may act like theaflavins to inhibit FAS activity.

It has been demonstrated that dietary green tea catechins increased fecal excretions of cholesterol and total lipids in cholesterol fed rats (*14*). Ikeda et al. also reported that tea catechins strongly lower cholesterol absorption from the intestine, especially EGCG and ECG, by reducing the solubility of cholesterol in mixed micelles (*28*). The content of catechins is higher in nonfermented green tea leaves than partially fermented oolong tea leaves and fully fermented black and pu-erh tea leaves in our analysis (**Table 3**), especially the level of (−)-epigallocatechin (EGC). Interestingly, the effects of the decreased body weight, serum triglyceride, total cholesterol, and LDL-C were greater in partially fermented oolong tea leaves, fully fermented black, and pu-erh tea leaves than in nonfermented green tea leave. Therefore, we propose two reasons to explain this phenomenon. First, the high level of (−)-epigallocatechin (EGC) in nonfermented green tea leaves might act to promote the lipid absorption. Another possible reason might be attributed to the process of the fermentation. It is well know that polyphenol oxidase in tea oxidized catechin into quinone and then condensed to form bisflavanol, theaflavin, thearubigen, and other high molecular components during the fermentation process (*6*). Hayakawa et al. also indicated that the high molecular weight fractions of four tea extracts had the ability to induce leukemia U937 and human stomach cancer MKN45 cells apoptosis (*29*). In **Table 3**, the total tea catechins content was greater in green tea than others, especially in fully fermented pu-erh tea. Yet pu-erh tea seemed to more efficiently lower the levels of serum triglyceride, relative weight of liver, and epididymal adipose tissue and increase the levels of HDL-C than green tea. Hayakawa et al. and our data indicated that some high molecular weight compound which could act as tea

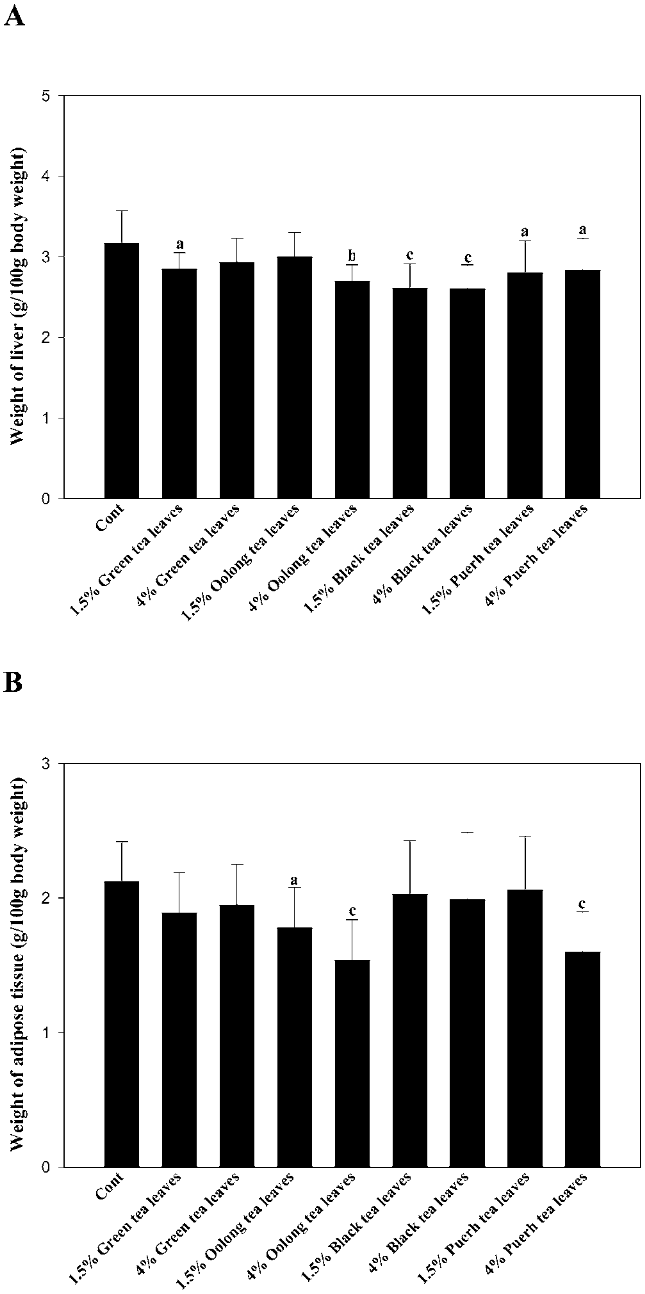

Figure 7. Effects of four tea leaves on the relative weight of liver and adipose tissue. Data are presented as the mean ± SE from 10 rats per group. Statistically different from the control group: (a) $p < 0.05$; (b) $p < 0.01$; (c) $p < 0.005$; (d) $p < 0.001$. (**A**) Liver, and (**B**) epididymal adipose tissue.

catechins but is more efficient than tea catechins was derived from catechins during the fermentative process. Previously our laboratory has been indicated that theaflavin, the major component of the fully fermented black tea leaves, could inhibit inflammatory protein (iNOS and COX-2) expression (*30*), rat liver microsomal 5-α reductase activity (*31*), and suppression of extracellular signals and cell proliferation (*32*) and fatty acid synthase (*2*). The polyphenol components in oolong tea leaves have not only induced tumor cell apoptosis (*33*, *34*) but also antibacterial activity (*35*). In another fully fermented pu-erh tea leave, it has the great radical scavenging activity and suppression of LPS-induced NO production in macrophage (*36*) and induce apoptosis activity (*29*). Based on these data and references, it is suggested that the great ability to decrease serum triglyceride, total cholesterol, and LDL-C may be contributed by the high molecular weight compounds which might be produced from the different stages of fermentation. These interesting unknown compounds deserve further exploration.

Finally, our results show that oral feeding of different degrees of fermentation tea leaves to SD rat results in the reduction of triglyceride, total cholesterol, and LDL-C and the enhancement of activities of SOD and HDL-C in serum. It is possible that this effect may refer to new high molecular weight polyphenols that are derived from catechins during fermentation. It may give a new direction in which to discover new hyprolipidemic compounds from those fermentative tea leaves.

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