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Effect of Oolong Tea Extracts on Plasma Glucose Level and Antioxidant System in Diabetic Rats

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ABSTRACT

The present study was conducted to investigate the effect of oolong tea extract on blood glucose level and antioxidant system in diabetic rats. The Sprague-Dawley rats were fed on AIN-76 based experimental diets containing 1% oolong tea extract for 6 weeks. They were induced to be diabetic by receiving streptozotocin (45mg/kg BW) intramuscularly. Blood glucose, blood and hepatic concentration of vitamins A and E, and antioxidant enzyme activities were measured. Oolong tea extract feeding decreased the plasma glucose in diabetic rats. Dietary supplementation of oolong tea extract did not affect antioxidative enzyme activities such as superoxide dismutase, glutathione peroxidase and catalase in diabetic rats. The plasma level of retinol was increased in diabetic rats by feeding oolong tea extract. Plasma and hepatic levels of α-tocopherol were higher in diabetic rats fed oolong tea extract. In conclusion, these results suggest that oolong tea extract consumption might reduce the plasma glucose in diabetic rats and protect the oxidative damage from diabetic stress to some extent. (*J Community Nutrition* 8(4): 207~213, 2006)

KEY WORDS: oolong tea · diabetic rats · glucose · antioxidant system.

Introduction

Diabetes mellitus is a chronic disease associated with serious complications. Atherosclerosis accounts for some 80% of all diabetic mortality; about three-quarters of the cardiovascular deaths from diabetes result from coronary artery disease (Nesto et al. 2001). Lipid oxidative damage can contribute to the occurrence and progression of vascular disease (Langseth 1995).

Polyphenols, the plant metabolites that are found in many different plant foods, have been shown to quench reactive oxygen species and chelate metal ions in vitro and in vivo (Lin et al. 1996). Tea is rich in polyphenols which have a wide range of biological and pharmacological benefits (Su et al. 2006). Tea is the most widely consumed beverage in the world. It can be categorized into three types, depending on the level of fermentation, i.e., green (unfermented), oolong (partially fermented), and black (fermented) tea (Cheng 2006).

Resent epidemiological studies have strongly suggested an inverse relationship between tea consumption and cardiovascular disease risk (Sesso et al. 1999). The green tea flavonoid has been shown to have insulin-like activities (Waltner-Law et al. 2002) as well as insulin-enhancing activity (Anderson 2002). While the antioxidative properties of green and black tea have been extensively studied, less attention has been given these properties in oolong tea. Oolong is commercially available in the United States, China, Japan, Korea and elsewhere, and is served in Chinese restaurants around the world. Oolong tea is semi-fermented to maintain a moderate level of enzymatic oxidation during processing and drying (Harbowy, Balentine 1997). In Japan (Shimada et al. 2004) and Taiwan (Hosoda et al. 2003), oolong tea was shown to be an effective adjunct to oral hypoglycemic agents in the treatment of patients with type 2 diabetes. Furthermore, oolong tea, in conjunction with antihyperglycemic agents, was more effective in lowering plasma glucose than were the drugs alone.

Xie et al. (1993) reported that oolong tea water extract had a high antioxidative activity and a high lipoxygenase inhibitory activity, as compared to black tea. In addition, Zhu et al. (2002) showed that 100g of oolong tea leaves produce appro-

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ximately 4.5g tea catechines, which contain 24.1% epigallocatechin, 3.5% catechin, 6.4% epicatechin, 41.8% epigallocatechin gallate, 13.5% gallocatechin gallate, and epicatechin gallate. The strong antioxidative activity of oolong tea fraction can possibly be attributed to the tea catechins present in oolong tea because their molecular weights are lower than 500. The strong antioxidative activity of oolong tea fraction is suggestive for the presence of lower molecular weight polymerized tea polyphenols in oolong tea (Hibasami et al. 2000). However, some researchers have shown that consumption of oolong tea elevates the metabolic rate and increases fat oxidation (Rumpler et al. 2001). Therefore we conducted this study to investigate the effect of dietary supplementation of oolong tea extract on blood glucose and antioxidative system in diabetic rats.

Materials and Methods

1. Animals and diets

Forty male Sprague-Dawley rats (Biogenomics Com., Korea) weighing 200 − 220g were housed individually in stainless steel mesh cages in a room at 22 ± 2℃ and 60 − 65% relative humidity with normal 12hr light-dark cycles. Rats were fed chow diet (Jinyang Co., Korea) and tap water *ad libitum* for 1 week before the experiment. Casein, vitamin and mineral mixtures for diets were purchased from Harlan Terklad Company (Madison, WI, USA).

After 1 week of acclimation, rats were randomly divided into four equal groups (n = 10 each). The four groups are described in Table 1. The dietary treatment consisted of AIN76-based diets (American Institute of Nutrition 1977) with or without oolong tea extract. On the 14th day after introduction of these diets, rats in diabetic groups received intramuscular injections of streptozotocin (STZ, Sigma, St. Louis, USA; 45 mg/kg BW in 0.1M citrate buffer, pH 4.5) to induce diabetes. The other rats received an injection of citrate buffer without STZ. Diabetic and normal rats were returned to their diets for 4 more weeks (for a total of six weeks on their respective diets). Table 1 represents the experimental design related to the dietary supplementation of oolong tea extract and STZ injection.

Table 1. Classification of experimental group (g/kg diet)

Group	Basal diet	Oolong tea[1)]	Streptozotocin[2)]
Normal	AIN-76	−	−
Normal + oolong	AIN-76	10	−
Diabetic	AIN-76	−	+
Diabetic + oolong	AIN-76	10	+

[1)] Water extract of oolong tea
[2)] Streptozotocin (45mg/kg BW) was injected to rats intramuscularly

A single source of oolong tea (*C. sinensis*) was obtained from Sumihun Tea Corporation (Seoul, Korea) and it was produced in Angye (Bockun province, China). Dry tea leaves were powdered in ceramic bowl. The powdered tea leaves were treated with 20 parts of distilled water and refluxed for 1 hour at 82℃. The extract was filtered through Whatman No. 1 filter paper, and concentrated by rotary evaporator (Buchi R-124, Switzerland). The condensed sample was dried by a freeze dryer (EYELA FD-1, Tokyo, Japan), and powdered to 50mesh. The yield of oolong tea extract was 37.7%. The powdered sample was contained in the experimental diets for rats.

2. Sample preparation

All animals were sacrificed 14 days after the STZ injection. Blood was drawn from the abdominal aorta into heparinized syringes, and plasma was obtained by centrifugation at 1,400g at 4℃ for 15 min. Livers were perfused with cold normal saline, then excised. After excision, livers were blotted dry, weighed, then frozen in liquid nitrogen. Liver homogenates were prepared at a ratio of 1g of wet tissue to 9mL of 1.15% KCl in a Teflon Potter-Elvehjem homogenizer. Mitochondria, microsomes, and cytosol were prepared according to the method of Hogeboom (Hogeboom 2000). Mitochondrial and microsomal fractions were finally suspended in 1.15% KCl, at a concentration of approximately 1mg of protein in a 0.1-mL suspension. Prepared samples were stored at −80℃ for analysis.

3. Biochemical analysis

Plasma glucose in rats was determined by the glucose oxidase method using a commercially available enzymatic kit.

Superoxide dismutase activity in plasma was determined by the method of Marklund, Marklund (Marlund, Marlund 1974). Enzyme activity inhibiting 50% of autoxidation of pyrogallol solution was defined as one unit of superoxide dismutase activity. Catalase activity in hepatic mitochondrial fractions was measured using the method of Aebi (Aebi 1974). The GSH-Px activity was measured by a modified method of Paglia and Valentine (Paglia, Valentine 1967). The activity was expressed as micromoles of oxidized NADPH per minute per milligram of protein. The G6Pase activity was measured by spectrophoto-

metric method (Baginski et al. 1983). The activity was expressed as nanomoles of released inorganic phosphorus per minute per milligram of protein. The protein concentrations of the hepatic subfractions used for the assays of antioxidant enzyme activities were measured by the method of Lowry et al. (Lowry et al. 1951) with bovine serum albumin as the standard.

Simultaneous determinations of plasma retinol and tocopherol were by high performance liquid chromatography using a modified method from Bieri et al. (Bieri et al. 1979). Two hundred μl of plasma was mixed with the same volume of retinyl acetate solution (80 μg/ml) and tocopheryl acetate solution (100 μg/ml) as internal standards. Blood lipid was thoroughly extracted with spectrograde hexane. The vials of hexane extracts were centrifuged to separate phases. The hexane extracts were filtered through a 0.45 μm nylon membrane into a small vial and evaporated under a stream of nitrogen. The dried extract was dissolved in chromatographic solvent (petroleum ether: methanol = 1/1, v/v). Twenty μl of the reconstituted sample was injected onto the HPLC (Serum: Shimadzu, Japan; Liver: Waters 6000A, USA) equipped with C_{18} Bondapack cartridge column (30cm × 3.9mm, 10 μm). The mobile phase in serum analysis was a mixture of methanol and water (95 : 5, v/v). Whereas the mobile phase in liver analysis was eluent A-methanol: H_2O (90 : 10) and eluent B-methanol : THF : H_2O (65 : 30 : 5) by modifying the method of Furr et al. (1984). Retinol and α-tocopherol were measured with UV detection at 280nm.

4. Statistical analysis

Results are presented as means and standard deviations. Group means were compared by Duncan's multiple range test after preliminary analysis of variance (ANOVA) and differences were considered statistically significant at $p < 0.05$. All statistical tests were performed using the computer software program SPSS for Windows (SPSS, Chicago, IL, USA; version 10.0) for statistics and data analyses.

Results

Fig. 1 shows the body weight in diabetic rats after STZ injection. The body weight gain of nondiabetic rats was not significantly different among groups. But oolong tea extract feeding increased the body weight gain in diabetic rats, even though it was lower than in nondiabetic rats.

Fig. 2 shows the effect of oolong tea extract on plasma level of glucose in normal and diabetic rats. Plasma glucose was significantly increased in diabetic rats compared with the normal rats ($p < 0.05$). Oolong tea extract feeding did not affect plasma glucose level in nondiabetic rats. Dietary supplementation with water extract of oolong tea resulted in lower plasma glucose in diabetic rats ($p < 0.05$).

As shown in Table 2, superoxide dismutase activity of diabetic rats was decreased compared with normal rats ($p < 0.05$). However, oolong tea extract feeding did not change the en-

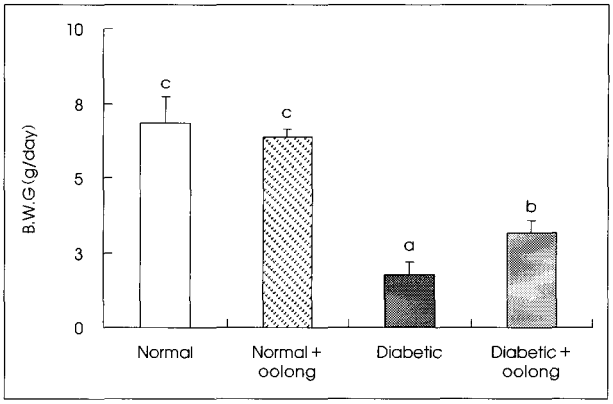

Fig. 1. Effect of oolong tea extract on the body weight gain in normal and diabetic rats after streptozotocin injection.

1) Means ± S.D. of 10 rats per group.
2) Rats of oolong tea groups were fed the water extract of oolong tea (10g/kg diet) for 6 weeks.
3) To induce diabetes, streptozotocin (45mg/kg BW) was injected to rats intramuscularly.
4) Values with the same superscript letter are not significantly different ($p < 0.05$).

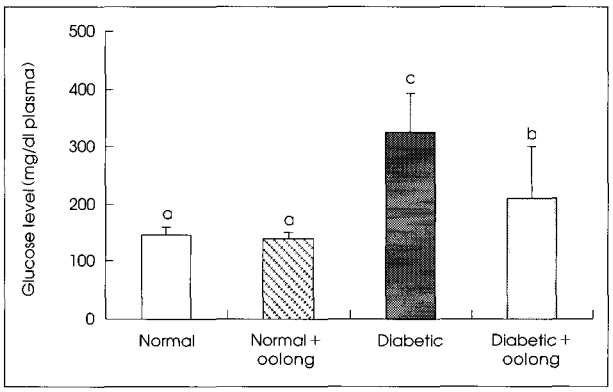

Fig. 2. Effect of oolong tea extract on plasma level of glucose in normal and diabetic rats.

1) Means ± S.D. of 10 rats per group.
2) Rats of oolong tea groups were fed the water extract of oolong tea (10g/kg diet) for 6 weeks.
3) To induce diabetes, streptozotocin (45mg/kg BW) was injected to rats intramuscularly.
4) Values with the same superscript letter are not significantly different ($p < 0.05$).

Table 2. Effect of oolong tea extract on SOD, Catalase, GSH-Px and G6Pase in liver of normal and diabetic rats

Group	SOD	Catalase	GSH-Px	G6Pase
		unit/min/mg protein		
Normal	0.19 ± 0.02[b]	81.2 ± 29.0[NS]	272.1 ± 58.4[NS]	0.70 ± 0.12[NS]
Normal + oolong	0.20 ± 0.02[b]	64.2 ± 26.9	290.3 ± 30.4	0.80 ± 0.08
Diabetic	0.17 ± 0.01[a]	54.7 ± 19.1	274.1 ± 55.9	0.76 ± 0.15
Diabetic + oolong	0.16 ± 0.01[a]	80.2 ± 11.9	293.6 ± 65.6	0.80 ± 0.15

[1] Means ± S.D. of 10 rats per group
[2] Rats of oolong tea groups were fed the water extract of oolong tea (10g/kg diet) for 6 weeks
[3] To induce diabetes, streptozotocin (45mg/kg BW) was injected to rats intramuscularly
[4] NS: Not significantly
[5] Values with the same superscript letter within the column are not significantly different ($p < 0.05$)

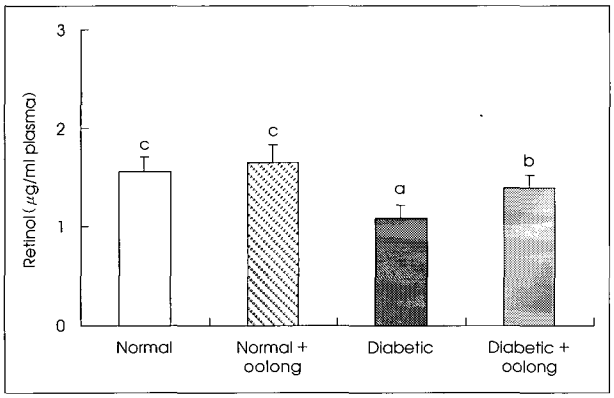

Fig. 3. Effect of oolong tea extract on plasma level of retinol in normal and diabetic rats.

[1] Means ± S.D. of 10 rats per group.
[2] Rats of oolong tea groups were fed the water extract of oolong tea (10g/kg diet) for 6 weeks.
[3] To induce diabetes, streptozotocin (45mg/kg BW) was injected to rats intramuscularly.
[4] Values with the same superscript letter are not significantly different ($p < 0.05$).

zyme activity in normal and diabetic rats. Catalase, glutathione peroxidase and glutathione-6-phosphatase activities were not different among all groups (Table 2).

As shown in Fig. 3, plasma level of retinol was increased in diabetic rats by feeding oolong tea extract ($p < 0.05$). However, dietary supplementation of oolong tea extract did not affect the plasma concentration of retinol in normal rats. Table 3 shows the effect of oolong tea extract on liver contents of vitamin A in normal and diabetic rats. Hepatic level of retinol was not different among the experimental groups. Hepatic concentration of retinyl palmitate tended to be lower in diabetic rats compared with the normal rats ($p < 0.05$). Oolong tea extract feeding increased the content in diabetic rats, but not significantly. Hepatic concentration of total vitamin A in diabetic rats without oolong tea extract was decreased significantly compared to the normal rats with oolong tea extract ($p < 0.05$). However, oolong tea extract feeding did not give the significant influence on total vitamin A concentration of normal and diabetic rats (Table 3).

Fig. 4 shows the effect of oolong tea extract on plasma and liver levels of α-tocopherol in normal and diabetic rats. Dietary supplementation of oolong tea extract did not affect the level in normal rats. However, plasma and hepatic levels of α-tocopherol were higher in diabetic rats fed oolong tea extract than in diabetic rats without oolong tea extract ($p < 0.05$).

Discussion

The development of metabolic syndrome such as diabetes mellitus and obesity is greatly influenced by life style (Morriss et al. 2005). The prominent risk factors of chronic diseases are diet-related. Although the classic essential nutrients are still widely studied, interest in evaluating the effects of bioactive compounds in foods on health is increasing. Even in a balanced diet that meets macronutrient recommendations and micronutrient requirements, there is a growing body of evidence that bioactive compounds play an important role in optimizing health (Kris-Etherton, Keen 2002). Flavonoids, one bioactive compound of foods, are an important group of phenolic compounds in plants. Tea is a rich sources of flavonoids. Moreover, there is some evidence that flavonoids derived from tea are more active than the homologous compounds from vegetables such as onions and spinach (Trevisanato, Kim 2000). Catechins are a major component of the green tea and oolong tea extracts. Yang et al. (2001) reported that almost half of these tea extracts were mixtures of catechins. In contrast, monomeric catechins only constituted less than 10% of the black tea extract. The sum of epigallocatechin and epigallocatechin gallate weighed more than 70% of the catechin mixture in the green and oolong tea extracts, and only half the

black tea extract. Tea catechin as a mixture and the individual epicatechin derivatives can be absorbed and circulated into the blood, where they may function as antioxidants protecting LDL from oxidation either directly, by protecting LDL from the attack of free radicals, or via the mechanism of maintaining and regenerating α-tocopherol.

In this study, dietary supplementation with water extract of oolong tea resulted in lower plasma glucose in diabetic rats ($p < 0.05$). Hosoda et al. (2003) reported that the plasma glucose concentration of diabetes patients decreased significantly after drinking oolong tea, but did not change after drinking water. In addition, oolong tea decreased the plasma levels of hemoglobin A1c. Shimada et al. (2004) suspected the mechanism for this result might be related to insulin resistance. There are some studies that have shown the insulin-like activity of tea polyphenols in rats (Broadhurst et al. 2000) and the delay of glucose absorption by tea in rats (Kreydiyych et al. 1994).

Even though some health benefits of tea have been known for many years, scientific studies of biological activities were started only recently. Among these studies, antioxidative chemicals have received much attention by many researchers because ingestion of these substances helps to prevent in vivo oxidative damage, such as lipid peroxidation, associated with many diseases including atherosclerosis, diabetes and cancer (Yanagimoto et al. 2003). However, the mechanism of the antihyperglycemic effect of oolong tea is not yet clear. The active components are not known, but both catechins and polysaccharides have been postulated as the active antidiabetic components (Anderson, Polansky 2002).

Diabetes mellitus and diabetic complications are associated with high oxidative stress levels, resulting from an imbalance between the production of free radicals or reactive oxygen species (ROS) and the antioxidant systems (Maritim et al. 2003). Oxidative stress is defined as excessive production of ROS in the presence of diminished antioxidant substances. It has been shown that oxidative stress has an adverse effect on glucose metabolism. Development of the chronic complications of diabetes mellitus has also been attributed to oxidative stress (Zhu et al. 2002). Therefore, tissue antioxidants helped prevent the cellular damage caused by free radicals and free radical-mediated lipid peroxidation (Henning et al. 1991).

Therefore we measured the antioxidative enzyme activities of diabetic rats to evaluate the effect on the antioxidant system after feeding oolong tea extract. Superoxide dismutase activity of diabetic rats was decreased compared with normal rats ($p < 0.05$). However, oolong tea extract feeding did not change the enzyme activity in normal and diabetic rats. Catalase and glutathione peroxidase activities were not different among all groups (Table 2).

The human body has several innate defense systems against reactive oxygen species. However, the innate oxidant defense system is not sufficient to adequately deal with the amount of

Table 3. Effect of oolong tea extract on liver contents of vitamin A in normal and diabeic rats

Group	Retinol	Retinyl palmitate	Total vitamin A
	$\mu g/g$	mg/g	$\mu mol/g$
Normal	118.37 ± 46.34[NS]	0.57 ± 0.12[ab]	1.51 ± 0.37[ab]
Normal + oolong	165.48 ± 30.53	0.68 ± 0.27[b]	1.88 ± 0.58[b]
Diabetic	188.38 ± 96.01	0.39 ± 0.07[a]	1.39 ± 0.35[a]
Diabetic + oolong	155.15 ± 41.36	0.56 ± 0.19[ab]	1.61 ± 0.30[ab]

[1] Means ± S.D. of 10 rats per group
[2] Rats of oolong tea groups were fed the water extract of oolong tea (10g/kg diet) for 6 weeks
[3] To induce diabetes, streptozotocin (45mg/kg BW) was injected to rats intramuscularly
[4] NS: Not significantly
[5] Values with the same superscript letter within the column are not significantly different ($p < 0.05$)

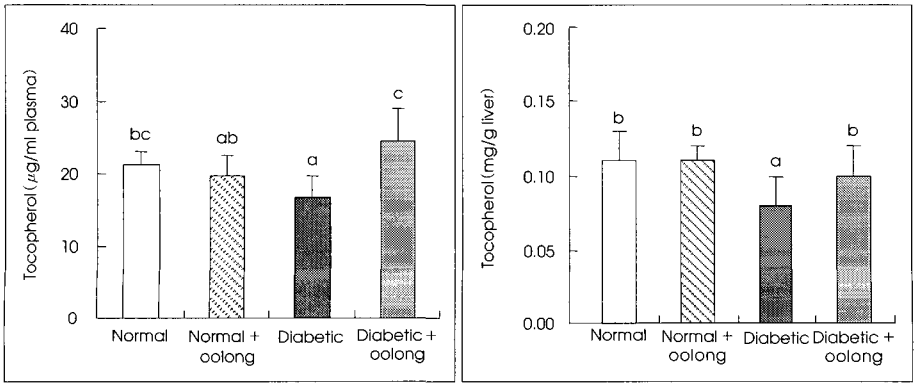

Fig. 4. Effect of oolong tea extract on plasma and liver levels of α-tocopherol in normal and diabetic rats. [1] Means ± S.D. of 10 rats per group, [2] Rats of oolong tea groups were fed the water extract of oolong tea (10g/kg diet) for 6 weeks, [3] To induce diabetes, streptozotocin (45mg/kg BW) was injected to rats intramuscularly, [4] Values with the same superscript letter are not significantly different ($p < 0.05$).

reactive oxygen species produced. It is now thought that dietary antioxidants are essential for health. In addition to antioxidant nutrients, several other botanical compounds such as flavonoids are thought to function as antioxidants and have been linked to a reduced incidence of diseases (Hertog et al. 1993). Zhu et al. (2002) reported oolong tea contained several low molecular weight antioxidants that might have health promotion activities.

Epidemiologic studies suggested an inverse association of tea consumption with cardiovascular disease. The antioxidant effects of flavonoids in tea are among the potential mechanisms that could underline the protective effects (Kris-Etherton, Keen 2002). Xie et al. (1993) reported that a water extract of oolong tea had higher antioxidative activity than black tea. Zhu et al. (2002) reported that the extract from 100g dry oolong tea using boiling water (1L) had greater antioxidant and free radical scavenging activity than 0.02% (w/w) butylated hydroxyanisole (BHA). Vitamin E, the major biological antioxidant, is probably the most important dietary free radical and lipid peroxide quencher due to its lipid solubility and occurrence in membranes (Tappel et al. 1973). We found that plasma and hepatic levels of α-tocopherol were higher in diabetic rats fed oolong tea extract than in diabetic rats without oolong tea extract ($p < 0.05$). Even though antioxidative enzyme activities were not changed significantly, plasma and hepatic levels of α-tocopherol, major antioxidant nutrient, were increased in diabetic rats by oolong tea extract. Serafini et al. (1996) reported that ingestion of tea beverage immediately produced a significant increase in human plasma antioxidant capacity in vivo.

As mentioned above, it is thought that the main cardiovascular effects of flavonoids may come from their anti-oxidant properties (Ryu et al. 2006). But more research is needed to see if tea drinking should be a standard recommendation for those who have diabetes or are at risk for developing diabetes (Cheng 2006).

In conclusion, this study supports the concept that oolong tea is effective in lowering the plasma glucose level of diabetic rats. Dietary supplementation of oolong tea extract increased plasma and hepatic levels of α-tocopherol in diabetic rats. So far, only a limited number of human intervention studies regarding the biological activity of oolong tea have been reported, and further clinical studies should be conducted. These studies should clarify the role of oolong tea in the promotion of human health and for disease prevention. Therefore, we need further studies to link to human study and to clarify the mechanisms.

Summary and Conclusion

The present study was conducted to investigate the effect of oolong tea extract on blood glucose level and antioxidant system in diabetic rats. The Sprague-Dawley rats were fed on AIN-76 based experimental diets containing 1% oolong tea extract for 6 weeks. They were induced to be diabetic by receiving streptozotocin (45mg/kg BW) intramuscularly. Oolong tea extract feeding increased the body weight gain in diabetic rats, even though it was lower than in nondiabetic rats. Oolong tea extract feeding decreased the plasma glucose in diabetic rats. Superoxide dismutase activity of diabetic rats was decreased compared with normal rats. However, oolong tea extract feeding did not change the enzyme activity in normal and diabetic rats. Catalase, glutathione peroxidase and glutathione-6-phosphatase activities were not different among all groups Plasma level of retinol was increased in diabetic rats by feeding oolong tea extract. Hepatic level of retinol was not different among the experimental groups. Plasma and hepatic levels of α-tocopherol were higher in diabetic rats fed oolong tea extract than in diabetic rats without oolong tea extract. In conclusion, these results suggest that oolong tea extract consumption might reduce the plasma glucose in diabetic rats and protect the oxidative damage from diabetic stress to some extent.

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