

The Effect of Green Tea on the Oxidative Stress and Blood Glucose Level of Diabetic Rats

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Abstract. Green tea was reported to have an antioxidant effect. However, few studies were found on the effect of green tea on the oxidative stress caused by diabetes mellitus. The aim of this study was to investigate the effect of green tea on the blood levels of lipid peroxidation product-malondialdehyde, superoxide dismutase enzyme and glutathione peroxidase enzyme. The effect of green tea on the blood glucose level of diabetic rats was also investigated. Three groups were examined in this study; they were a control group, a diabetic group and a diabetic group who used green tea. The blood level of malondialdehyde, superoxide dismutase enzyme and glutathione peroxidase enzyme were measured at 6 and 12 weeks after the induction of diabetes and the use of green tea. The blood glucose levels of the three groups were also measured at 6 and 12 weeks. The results obtained from this study showed that the use of green tea reduced the level of malondialdehyde significantly, and it caused a noticeable increase in the activity of superoxide dismutase and glutathione peroxidase enzymes. It also clearly reduced the level of blood glucose of diabetic rats.

Keywords: Green tea, Malondialdehyde, Superoxide dismutase, Glutathione peroxidase, Diabetes, Rats.

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Introduction

Tea is a widely used beverage in the world. Green tea was reported to have many pharmacological actions including antioxidant, antipyretic, antibacterial and antineoplastic effects^[1-5]. Green tea contains polyphenols, which are also known as flavonoids or catechins. Polyphenols are responsible for the pharmacological actions of green tea^[1,6].

Green tea was reported to reduce oxidative stress caused by *diabetes mellitus* (DM), ethanol and ammonium metavanadate exposure^[5,7-10]. However, few studies on the antioxidant effect of green tea on oxidative stress of DM have been reported in literature. The effect of green tea on the blood glucose level in DM has been previously studied. However, the studies obtained from literature showed controversial results. Janle *et al.*^[11] found that green tea did not significantly lower the blood glucose level in diabetes, while Sabu *et al.*^[7] reported that green tea did lower the blood glucose level in diabetes.

The aim of this study was to investigate the antioxidant effect of green tea on the oxidative stress in diabetic rats. The levels of malondialdehyde (MDA), super oxide dismutase (SOD) and glutathione peroxidase (GSH Px) were measured. This investigation examined the effect of green tea on the blood glucose level of diabetic rats.

Materials and Methods

Animal Groups

Adult male Lewis albino rats aged 60 d and weighing 180-250 g were used. The rats were allowed free access to food and water. They were kept at a constant temperature of 24°C and a light cycle of 12 h on / 12 h off. The rats were made diabetic by a single intravenous injection of streptozotocin (Sigma, St. Louis, MO, USA). The dose of streptozotocin was 50 mg/kg. Non-fasting blood glucose concentrations were measured once daily for one week to confirm the presence of diabetes. Animals with a blood glucose level above 200 mg/dl were used in this study.

Three groups were studied in this investigation. Each group consisted of 12 rats. The control group consisted of non-diabetic rats who did not receive green tea. The second group consisted of diabetic rats. The third group consisted of diabetic rats who received green tea orally

after one week of induction of diabetes. The total number of studied rats was 36 rats and rats made diabetes were 24 rats. Green tea extract (0.5 g of lyophilized green tea powder dissolved in 100 ml of deionized distilled water) was given daily to the rats for 6 or 12 weeks. Green tea was prepared from steaming fresh *Camellia sinensis* leaves at a high temperature. The polyphenols found in tea are known as flavonoids or catechins and comprise 30-40% of the extractable solids of dried green tea leaves^[4]. The dose of green tea was 300 mg/kg/day. Blood samples were collected from the three groups and examined at 6 and 12 weeks after the induction of diabetes and the use of green tea. The blood level of MDA, SOD, and GSH Px were measured. The blood glucose levels for the three groups were also measured by using glucometer (Avantage, Boehringer Mannheim, IN, USA).

Biochemical Analysis

The blood level of MDA was measured according to the method of Ohkawa *et al.*^[12]. The level of SOD was measured according to the method of Misra and Fridovich^[13]. The GSH Px level was measured by using a RANSEL kit (RANDOX Laboratories Ltd., UK).

Statistical Analysis

All results were expressed as mean \pm SD. The data were analyzed statistically by one way analysis of the variance (ANOVA) and Bonferroni tests. All the statistical computations were made using the statistical packages, Statistical Package for the Social Sciences (SPSS) Version 10.0 for Microsoft Windows and Microsoft Excel (2002). The difference was considered as significance when $p < 0.05$.

Results

The administration of green tea lowered the blood glucose level of the diabetic group significantly at 6 and 12 weeks. The blood glucose levels of the control group were 97.12 ± 13.74 mg/dl and 84 ± 14.9 mg/dl at 6 and 12 weeks, respectively. The blood glucose levels of the diabetic group were 376.3 ± 91.2 mg/dl and 410 ± 30 mg/dl at 6 and 12 weeks, respectively. At 6 and 12 weeks, the blood glucose levels of the diabetic group using green tea were 330 ± 24 mg/dl and 295 ± 15 mg/dl (Table 1).

Table 1. The blood glucose level of control, streptozotocin (STZN) induced diabetic group and streptozotocin (STZN) induced diabetic group using green tea.

Duration of Treatment	Blood glucose level (mg/dl)	
	6 th wk	12 th wk
Control	97.12 ± 13.74	84 ± 14.9
STZ-induced DM	376.3 ± 91.2*	410 ± 30*
STZ-induced DM and Green Tea	330 ± 24*†	295 ± 15*†

Values are represented as mean ± SD (n = 6).

Significance was considered as P < 0.05.

*Significance compared to the control.

†Significance compared to the corresponding diabetic group.

At 6 and 12 weeks, the blood levels of lipid peroxidation product-MDA of the control group were 2.59 ± 0.6 nmol/ml and 2.7 ± 0.3 nmol/ml, respectively. The blood levels of MDA of the diabetic group were 4.38 ± 0.15 nmol/ml and 5.5 ± 0.9 nmol/ml; while, the levels of MDA of the diabetic group using green tea were 3.2 ± 0.7 nmol/ml and 2.9 ± 0.3 nmol/ml. The levels of MDA of the diabetic group using green tea were significantly lower than those of the diabetic group at 6 and 12 weeks (Table 2).

Table 2. The blood level of malondialdehyde, superoxide dismutase enzyme and glutathione peroxidase of control, streptozotocin (STZ) induced diabetic group and streptozotocin (STZ) induced diabetic group using green tea.

Duration of treatment	Malondialdehyde (MDA) (nmol/ml)		Superoxide dismutase (SOD) (U/gHb)		Glutathione peroxidase (GSH Px) (U/gHb)	
	6 th wk	12 th wk	6 th wk	12 th wk	6 th wk	12 th wk
Control	2.59 ± 0.6	2.7 ± 0.3	1200 ± 60	1250 ± 40	250 ± 25	254 ± 17
STZ-induced DM	4.38 ± 0.15*	5.5 ± 0.9*	800 ± 45.7*	650 ± 90*	170.4 ± 19*	130 ± 22*
STZ-induced DM and green tea	3.2 ± 0.7*†	2.9 ± 0.3†	950 ± 66*†	1210 ± 30†	190.9 ± 8.5*†	210 ± 10*†

Values are represented as mean ± SD (n = 6). Significance was considered as P < 0.05.

*Significance compared to the control.

†Significance compared to the corresponding diabetic group.

The blood levels of SOD of the control group were 1200 ± 60 U/gHb and 1250 ± 40 U/gHb at 6 and 12 weeks, respectively. The levels of SOD of the diabetic group were 800 ± 45.7 U/gHb and 650 ± 90 U/gHb at 6 and 12 weeks, respectively; while the levels of SOD of the diabetic group using green tea were 950 ± 66 U/gHb and 1210 ± 30 U/gHb at 6 and 12 weeks, respectively. The levels of SOD of the diabetic group using green tea were significantly higher than those of the diabetic group at 6 and 12 weeks, respectively (Table 2).

At 6 and 12 weeks, the blood levels of GSH Px of the control group were 250 ± 25 U/gHb and 254 ± 17 U/gHb, respectively. The levels of GSH Px of the diabetic group were 170.4 ± 19 U/gHb and 130 ± 22 U/gHb; while the levels of GSH Px of the diabetic group using green tea were 190.9 ± 8.5 U/gHb and 210 ± 10 U/gHb. The levels of GSH Px of the diabetic group using green tea were significantly higher than those of the diabetic group at 6 and 12 weeks (Table 2).

Discussion

The results obtained from this study showed that the induction of diabetes in rats caused a significant increase in serum concentration of lipid peroxidation product-MDA, and it caused a significant decrease in plasma activity of antioxidant enzymes, which are SOD and GSH Px.

The administration of green tea in the diabetic group caused a decrease in MDA concentration and increased the activity of SOD and GSH Px enzymes. The MDA concentration in the diabetic group using green tea was significantly lower than that of the diabetic group at 6 and 12 weeks. The activities of SOD and GSH Px enzymes in the diabetic group using green tea were significantly higher than those of the diabetic group.

These results suggest that the administration of green tea reduces the lipid peroxidation and oxidative stress found in diabetic rats. Green tea was found to have an antioxidant effect^[6,10,14-16]. However, few studies were found in literature on the effect of green tea on the oxidant stress induced by DM. Frei and Higdon^[6] reported that green tea has an antioxidant effect in rats. Mori and Hasegawa^[17] reported that green tea causes an increase in the activity of SOD in rats. Hirano-Ohmori *et al.*^[18] and Nagao *et al.*^[19] reported that consumption of green tea decreased

serum MDA modified LDL concentration in humans. Luczaj *et al.*^[14] and Augustyniak *et al.*^[10] found that green tea decreased the oxidative stress caused by ethanol administration in rats. They also reported that the administration of green tea increased the activity of SOD and GSH Px enzymes and decreased the level of MDA. Erba *et al.*^[15] found that green tea improved the antioxidant status and protected against oxidative damage in humans. Hamdaoui *et al.*^[16] reported that green tea showed an antioxidant effect and caused an increase in the blood level of GSH Px in rats. Coimbra *et al.*^[4] reported that green tea has an antioxidant effect in humans. They added that it lowered the level of MDA. Soussi *et al.*^[5] found that green tea improved the oxidative stress induced by ammonium metavanadate in rats. They also added that green tea caused an increase in SOD and catalytic activities.

Oxygen free radicals and related reactive species were produced during oxidative stress. They contributed to the etiology of many diseases, such as atherosclerosis, neurodegenerative disorders and cancer. Antioxidant enzymes were present in cells to protect against the damaging effects of these free radicals and other oxygen-derived species^[2].

Green tea contains polyphenols, which are also known as flavonoids or catechins. These polyphenols are believed to induce the antioxidant effect of green tea^[1,6,7]. Tea polyphenols act as antioxidants *in vitro* by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. They may also inhibit the redox-sensitive transcription factors and inhibit the pro-oxidant enzymes^[6].

In the present study, green tea reduced the blood glucose level of diabetic rats. The blood glucose level of the diabetic rats using green tea was significantly lower than that of the diabetic group at 6 and 12 weeks. These results suggest that green tea lowers the blood glucose level of diabetic cases. In literature, controversial results were reported. Janle *et al.*^[11] reported that green tea did not significantly lower the blood glucose level in rats. However, Sabu *et al.*^[7] reported that green tea has an antioxidant effect and lowered the blood glucose level in rats. They also added that green tea improved SOD in diabetic rats but did not affect GSH Px or catalytic enzymes. Han^[20] found that green tea prevented the destruction of pancreatic B cells and prevented the progress of DM in rats. Wu *et al.*^[21] reported that green tea improved hyperglycemia,

hyperinsulinemia, and glucose uptake by adipocytes in hyperglycemic rats. Tsuneki *et al.*^[22] found that green tea promoted glucose metabolism and lowered the blood glucose level of diabetic mice.

In conclusion, this investigation showed that the administration of green tea reduced lipid peroxidation and caused an increase in the activity of antioxidant enzymes in diabetic rats. This would help in relieving the oxidative stress caused by DM. Green tea also reduced the blood glucose level of diabetic rats.

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تأثير الشاي الأخضر على الإجهاد التأكسدي المصاحب لداء السكري و مستوى السكر في الدم للجرذان المصابة بالسكري

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المستخلص. إن الشاي الأخضر مفعولاً مضاداً للأكسدة ولكن دراسات قليلة وجدت حول تأثير الشاي الأخضر على الإجهاد التأكسدي المصاحب لداء السكري. و لقد كان الغرض من البحث دراسة تأثير الشاي الأخضر على مستويات المالونديالدهيد والسوبر أكسيد ديسميوتاز والجلوتاثيون بروكسيداز. بالإضافة إلى دراسة تأثيره على مستوى السكر في الدم للجرذان المصابة بالسكري. ولقد تمت الدراسة على ثلاث مجموعات هي: مجموعة السيطرة غير المعالجة، والمجموعة المصابة بالسكري، والمجموعة المصابة بالسكري والتي تم إعطاؤها الشاي الأخضر. ولقد تم قياس مستويات المالونديالدهيد والسوبر أكسيد ديسميوتاز والجلوتاثيون بروكسيداز في الدم للمجموعات الثلاث، وفي الأسبوع السادس والأسبوع الثاني عشر بعد إحداث إصابة المجموعتين الثانية والثالثة بالسكري. وقد أظهرت نتائج الدراسة أن تناول الشاي الأخضر أدى إلى انخفاض واضح في مستوى مادة المالونديالدهيد، وفي مستوى السكر في الدم في جرذان المجموعة الثالثة، كما أدى أيضاً إلى زيادة نشاط أنزيم السوبر أكسيد ديسميوتاز وأنزيم الجلوتاثيون بروكسيديا في نفس المجموعة مقارنة بالمجموعتين الأولى والثانية.