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**Potential Impact of Green Tea on Public Health of Diabetic rats**

**Mohamed Moustafa El-Sayed, Khaled Aly Abd EL-Rahman Shaheen,  
Mohamed Fekri Serag El-Din, Mona Hamed Mohamed Moharm**

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**Abstract**

This study was carried out to evaluate the effect of different levels of boiled and non-boiled green tea as a source of anti-oxidant and phenolic compounds on body weight gain (BWG %), feed intake, feed efficiency ratio, lipid profile, glucose, Insulin and liver enzymes on diabetic rats. Thirty male albino rats were divided into two main groups; the first group (5 rats) fed basal diet used as negative control group. The second group (25 rats) diabetic rats. Where diabetes was induced by Streptozotocin (40 mg/Kg) in rats. Diabetic were divided into 5 sub-groups (5 per each) sub group (1) served as positive control group, sub group 2,3 were fed basal diet and given orally non-boiled green tea in a dose of 1.5 and 3 ml, respectively. Sub group 4, 5 were fed basal diet and given orally boiled green tea in a dose of 1.5 and 3 ml, respectively. The results indicated that treatments significantly decreased the levels of BWG, FER, FI, T.G, cholesterol, LDLc, VLDLc and liver enzymes (ALT and AST) as compared with positive control group. On the other hand, the treatments significantly increased HDL as compared with positive control. In conclusion, boiled and non boiled green tea can improve serum glucose level, lipid profile and liver function. All concentrations led to improvement.

**Keywords:** Green tea, diabetes mellitus, lipid profile, liver function.

## **1- Introduction**

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (*ADA, 2009*)

Diabetes mellitus is now considered to be a worldwide epidemic and without primary prevention, the epidemic will continue increasing. The complications of diabetes mellitus, like retinopathy, nephropathy and neuropathy, are results of such pathologic mechanisms. But these complications can significantly be prevented or their occurrence can be delayed by strict control blood glucose level (*Mirzaei et al., 2010; Vinson and Zhang, 2005*).

The World Health Organization Expert Committee on diabetes recommended that traditional medicinal herbs considered being less toxic and relatively free from side effects. In general, herbal medicines are complex mixtures of different compounds that often act synergistically to exert their full beneficial effect on diabetes mellitus and other disease (*Hussain and Marouf, 2013*).

Tea is one of the most widely consumed beverages worldwide, and is the second-most consumed drink after water (*Graham, 1992*). It is produced from the leaves, buds, or delicate stems of the plants of the genus *Camellia*. The most widely used plant species for tea is *Camellia Sinensis (L.) Kuntze*. Inhabitants of Europe, mainly Great Britain, are the largest consumers of tea (~540 mL) per day (*Gardner et al., 2007*). However, on average across the world population, a person consumes ~120 mL of tea per day (*Wierzejska, 2014*).

Green tea compounds may influence glucose metabolism by several mechanisms, such as inhibition of carbohydrate digestion and glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic B cells, modulation of glucose release from liver, activation of insulin receptors (enhancing insulin binding) and glucose uptake in the insulin sensitive tissues, and modulation of hepatic glucose output (*Hanhineva et al., 2010*). Therefore, this study aims to evaluate the effect of green tea on health of diabetic rats.

## **2- Materials and Methods**

### **2.1. Materials**

- Casein, all vitamins, all minerals, cellulose, Cholinechloride and Methionine and Streptozotocin (STZ, Sigma Chemical Company®, St. Louis, Millstone) were obtained from El-Gomhoria Company, Cairo, Egypt.
- The kits were supplied by Bio diagnostics company Cairo, Egypt.
- Green tea (*camellia sinensis*) was obtained from Agricultural Research Center.

### **2.2 Methods**

#### **2.2.1. Preparation of green tea:**

Dried green tea leaves (16 g) were added to 1 litre of deionised boiled water. The solution was kept to stand for 10 min before being filtered, cooled to room temperature, and dispensed in clean drinking bottles according to *Jankun et al., (1997)*.

#### **2.2.2. Induction of diabetes:**

After two weeks of acclimatization, the diabetes was induced in rats with Streptozotocin. Streptozotocin was intravenously (i.v.) administered in a dose of 40 mg/Kg dissolved in citrate buffer (0.1 M, pH 6.5). Control rats received citrate buffer. Seven days after diabetes induction, and glucose concentrations exceeded 300 mg/dL confirmed the diabetic state according by *Damascene et al., (2012)*.

#### **2.2.3. Experimental design:**

The experimental was done in the Faculty of Home Economics, Menoufiya University, Shebin El-kom. The animals were housed individually in well aerated cages under hygienic laboratory condition and fed standard diet according to AIN-93 guidelines (*Reeves et al., 1993*). For 7 days as an adaptation period. Thirty male albino rats were divided into two main groups; the first group (5 rats) fed basal diet used as negative control group. The second group (25 rats) was diabetic rats. Diabetic groups were divided into 5 sub-groups (5 per each) sub group (1) served as positive control group, sub group 2,3 were fed basal diet and given orally non-boiled green tea in a dose of 1.5 and 3 ml, respectively. Sub group 4, 5 were fed basal diet and given orally boiled green tea in a dose of 1.5 and 3 ml, respectively. During the experimental period, the body weight and feed intake were estimated weekly and the general behavior of rats was observed. At the end of the experimental period (28 days), each rat weight separately then, rats are slaughtered and collect blood samples. Blood samples were

centrifuged at 3000 rpm for ten minute to separate blood serum, and then kept in deep freezer (-20° C) till using.

#### **2.2.4. Biological evaluation:**

The body weight gain (**B.W.G.%**) , feed efficiency ratio (**F.E.R**), and organ/ body weight % were determined according to *Chapman et al., (1959)*. Using the following equations:

$$\text{B.W.G. (\%)} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}}$$

$$\text{F.E.R.} = \frac{\text{Grams gain in body weight}}{\text{Grams feed consumed}}$$

#### **2.2.5. Biochemical analysis:**

Serum total cholesterol, triglyceride (TG) and high density lipoprotein (HDLc) were determined by using methods of *Allain et al. (1974)*, *Fossati and Prencipe (1982)* and *Lopez-Virella (1977)*, respectively. The determination of low density lipoprotein cholestereol (LDLc) and very low density lipoproteins (VLDLc) were carried out according to the methods of *Lee and Nieman (1996)* as follows: VLDLc = TG/5 and LDLc = TC- (HDLc + VLDLc).

Determination of glucose and Insulin hormone were carried out according to the methods of *Young, (2001)* and *DeFronzo et al. (1979)* respectively. Alanine transaminase (ALT) activities were measured in serum using the modified kinetic method of *Tietz, (1976)* and Aspartate transaminase (AST) *Henry, (1974)* respectively.

#### **2.2.6. Statistical analysis:**

Data were statistically analyzed using statistical analysis system (*Armitage and Berry, 1987*). One way analysis of variance (ANOVA) was used to test the variations among groups and post Hoc test (Duncan's Test) was used to compare group means.

### **3- Result and Discussion**

Table (1) illustrate effect of different levels of boiled and non-boiled green tea on feed intake (FI), feed efficiency ratio and body weight gain % of diabetic rats. As shown the mean value of FI of positive control group was significantly lower than negative control group, which was 11.16g and 16.38g respectively, the mean value of groups feed on different levels of boiled and non-boiled green tea were significantly higher than positive control group. The best result was recorded for group 3 which fed on 1.5 ml non boiled green tea.

As for FER there was significant difference among negative control group and diabetic groups. Also, FER of groups feed on different levels of

boiled and non-boiled green tea were significantly higher than positive control group.

Concerning BWG%, the results indicated that the mean value of BWG (%) of positive control group was significantly lower than negative control group and diabetic group treated with boiled and non-boiled green tea. Adminstratis diabetic rats with 1.5 ml of non-boiled green tea was more effective ( $p < 0.05$ ) in improvement BWG than diabetic rats with different concentration of boiled and non-boiled green tea. These results are supported by the results published by *Wenping et al. (2013)* who reported that, green tea could effectively suppress the gain in body weight.

**Table (1): Effect of different levels of boiled and non-boiled green tea on Feed Intake, feed efficiency ratio and Body weight gain of diabetic rats**

Parameters	FI (g)	BWG (%)	FER (g)
<b>G(1): Control (-ve)</b>	16.38+1.42 <sup>a</sup>	16.76+1.53 <sup>a</sup>	0.076+0.01 <sup>a</sup>
<b>G(2): Control (±ve)</b>	11.16+1.13 <sup>g</sup>	4.22+1.15 <sup>j</sup>	0.022+0.03 <sup>j</sup>
<b>G(3):Non boiled green tea 1.5 ml</b>	15.11+1.17 <sup>b</sup>	11.25+1.75 <sup>b</sup>	0.057+0.01 <sup>f</sup>
<b>G(4):Non boiled green tea 3 ml</b>	14.13+1.83 <sup>d</sup>	9.31+1.11 <sup>d</sup>	0.063+0.03 <sup>d</sup>
<b>G(5):Boiled green tea 1.5 ml</b>	14.74+1.63 <sup>c</sup>	9.79+1.33 <sup>c</sup>	0.065+0.02 <sup>c</sup>
<b>G(6):Boiled green tea 3 ml</b>	13.89+1.91 <sup>e</sup>	8.19+1.46 <sup>h</sup>	0.069+0.04 <sup>b</sup>

- All values represented as mean ± SD. Means with different superscript letters in the same column are significantly different at  $p \leq 0.05$ .

Table (2) indicate the effect of different levels of boiled and non-boiled green tea on lipids profile of diabetic rats. The results recorded that the mean values of TC, TG, LDL and VLDL of positive control group were significantly higher than negative control group, which HDL had opposite trend. The mean values of TC, TG, LDL and VLDL of groups feed on different levels of boiled and non-boiled green tea were significantly lower than positive control group. The best result was recorded for group 6 which fed on 3 ml boiled green tea.

*Ramadan et al. (2009)* found that the high dose of green tea extract (GTE) completely reversed the increase in triglycerides

induced by alloxan and the cholesterol-rich diet. *Zheng et al. (2011)* showed that the administration of green tea beverages or extracts resulted in significant reductions in serum TC and LDL-cholesterol concentrations, but no effect on HDL cholesterol was observed. *Bursill and Roach (2007) and Bursill et al. (2001)* concluded that the administration of green tea extract was able to significantly increase both the LDL-receptor binding activity and relative amounts of LDL-receptor protein. In addition, there is another possible major mechanism by which green tea lowers cholesterol: catechins have direct inhibitory effects on cholesterol synthesis. *Abe et al. (2000)* showed that these effects of green tea are similar to hypocholesterolemic drugs such as statins, which reduce cholesterol synthesis and increase the LDL-receptor. *Hsu et al. (2008)* revealed that green tea intakes significantly decreased LDL-cholesterol concentrations and markedly increased concentrations of HDL cholesterol.

**Table (3): Effect of different levels of boiled and non-boiled green tea on lipids profile of diabetic rats.**

Parameters Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
G(1): Control (-ve)	94.75±2.43 <sup>j</sup>	61.77±1.25 <sup>j</sup>	47.73±1.83 <sup>a</sup>	34.67±1.44 <sup>j</sup>	12.35±1.39 <sup>j</sup>
G(2): Control (±ve)	161.22±5.15 <sup>a</sup>	98.53±3.91 <sup>a</sup>	26.89±2.23 <sup>j</sup>	114.63±5.36 <sup>a</sup>	19.70±1.33 <sup>a</sup>
G(3):Non boiled green tea 1.5 mg	138.17±3.75 <sup>e</sup>	85.23±2.33 <sup>e</sup>	32.62±2.63 <sup>h</sup>	88.51±4.15 <sup>e</sup>	17.04±1.15 <sup>e</sup>
G(4):Non boiled green tea 3 mg	131.99±2.44 <sup>h</sup>	79.66±1.14 <sup>g</sup>	36.14±3.17 <sup>e</sup>	79.92±1.35 <sup>g</sup>	15.93±1.11 <sup>g</sup>
G(5):Boiled green tea 1.5 mg	135.14±1.97 <sup>f</sup>	78.34±2.81 <sup>h</sup>	38.25±1.92 <sup>d</sup>	81.23±3.25 <sup>f</sup>	15.66±1.21 <sup>h</sup>
G(6):Boiled green tea 3 mg	121.63±1.36 <sup>i</sup>	74.16±2.11 <sup>i</sup>	41.33±1.83 <sup>b</sup>	65.74±2.12 <sup>i</sup>	14.83±1.33 <sup>i</sup>

- All values represented as mean ± SD. Means with different superscript letters in the same column are significantly different at p≤0.05.

Table (3) illustrate effect of different levels of boiled and non-boiled green tea on glucose and insulin of diabetic rats. The mean value of serum glucose level of positive control group was significantly higher than negative control group, which was 211.55 and 110.26 (mg/dl), respectively. Also, the mean values of treated groups with different levels of boiled and non-boiled green tea indicated a significant decrease in serum glucose compared with positive control. The best result was recorded for group 6 which fed on 3 ml boiled green tea.

Concerning insulin, the results indicated that the mean value of positive control group was significantly lower than negative control group, which 5.87 and 12.18 mg/dl respectively. The mean value of groups feed on different levels of boiled and non-boiled green tea were significantly higher than positive control group. The best result was recorded for group 6 which fed on 3 ml boiled green tea.

In agreement with these finding, *Wenping et al. (2013)* found that, green tea had remarkable hypoglycemic effects in type 2 diabetes. They suggested that green tea extract could improve the impaired glucose tolerance in diabetic CD-1 mice. *Wu et al. (2004)* showed that the alleviation of insulin resistance by green tea is associated with the increased expression level of Glucose transporter type 4 (GLUT4) in a fructose-fed rat model. *Cao et al. (2007)* showed that rats fed green tea at 1 or 2 g/kg diet regulates gene expression in the glucose uptake and insulin signaling pathway including glucose transporter, insulin receptor substrate genes.

**Table (3): Effect of different levels of boiled and non-boiled green tea on glucose and insulin of diabetic rats.**

Parameters Groups	Glucose (mg/dl)	Insulin (U/ml)
G(1):Control (-ve)	110.26+3.15 <sup>j</sup>	12.18+1.11 <sup>a</sup>
G(2):Control (±ve)	211.55+7.63 <sup>a</sup>	5.87+1.26 <sup>j</sup>
G(3):Non boiled green tea 1.5 mg	175.16+5.54 <sup>c</sup>	9.36+1.15 <sup>h</sup>
G(4):Non boiled green tea 3 mg	156.72+6.92 <sup>f</sup>	9.86+1.38 <sup>i</sup>
G(5):Boiled green tea 1.5 mg	153.11+1.17 <sup>g</sup>	10.61+1.45 <sup>c</sup>
G(6):Boiled green tea 3 mg	140.22+3.22 <sup>i</sup>	10.92+1.62 <sup>b</sup>

- All values represented as mean ± SD. Means with different superscript letters in the same column are significantly different at  $p \leq 0.05$ .

The Effect of different levels of boiled and non-boiled green tea on liver enzymes (ALT and AST) of diabetic rats are recorded in table (4).

As for **AST** , it could be noticed that the mean values of positive control group was significantly increased when compared to negative control group, which was 50.38 and 30.52 u/l, respectively. The mean value of groups feed on different levels of boiled and non-boiled green tea were significantly lower than positive control group. The best result was recorded for group 6 which fed on 3 ml boiled green tea. In agreement with these finding, *Raoofi et al. (2016)* found that green tea extract at 100, 200 and 400 mg/kg doses showed that a significant decline at aspartate transaminase (AST), alanine transaminase (ALT). *Korsandi et al. (2010)* found that green tea extract could significantly reduce ALT and AST in rats that received the insecticide Fenitrothion. *Ju-Hua et al. (2004)* concluded that taking green tea extract could significantly reduce ALT and AST levels in rats with liver failure induced by leflunomide.

Concerning **ALT**, the results indicated that the mean value of positive control group was higher than that of negative control group (healthy rats), which was 59.35 and 38.18 u/l. The mean value of groups feed on different levels of boiled and non-boiled green tea were significantly lower than positive control group. The best result was recorded for group 6 which fed on 3 ml boiled green tea.

**Table (4): Effect of different levels of boiled and non-boiled green tea on liver enzymes of diabetic rats**

Parameters	AST (U/L)	ALT (U/L)
<b>G(1): Control (-ve)</b>	30.52+1.18 <sup>j</sup>	38.18+2.38 <sup>j</sup>
<b>G(2): Control (±ve)</b>	50.38+3.32 <sup>a</sup>	59.35+3.76 <sup>a</sup>
<b>G(3):Non boiled green tea 1.5 mg</b>	45.12+2.33 <sup>e</sup>	51.34+3.39 <sup>c</sup>
<b>G(4):Non boiled green tea 3 mg</b>	38.79+2.77 <sup>h</sup>	43.74+2.75 <sup>h</sup>
<b>G(5):Boiled green tea 1.5 mg</b>	40.65+2.84 <sup>f</sup>	47.54+2.13 <sup>e</sup>
<b>G(6):Boiled green tea 3 mg</b>	36.24+3.34 <sup>i</sup>	40.27+2.92 <sup>i</sup>

- All values represented as mean ± SD. Means with different superscript letters in the same column are significantly different at p≤0.05.

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محمد مصطفى السيد ،خالد علي عبد الرحمن شاهين ،محمد فكرى سراج الدين،  
منى حامد محمد محرم

### الملخص العربي

أجريت هذه الدراسة لتقييم تأثير مستويات مختلفة من الشاي الأخضر المغلي والغير مغلي علي الفئران المصابة بالسكر لكونهم مصادر غنية بمضادات الأكسدة والمركبات الفينولية ودراسة تأثيرهم علي كل من معدل الوزن المكتسب والمأخوذ من الطعام ومعدل الإستفادة من الغذاء ودهون الدم ووظائف الكبد ومستوي كلا من الجلوكوزو الأنسولين بالدم. وقد تم استخدام ثلاثون فأر بالغ و تقسيمهم الي مجموعتين رأسييتين. ووضعت المجموعة الرئيسية الأولى كمجموعة ضابطة سالبة بينما المجموعة الرئيسية الثانية كانت مكونة من ٢٥ فأر وتم إصابتهم بالسكري بمادة الاستربتوزوكاسين (٤٠ ملجم/كجم) ثم تقسيمهم الي ٥ مجاميع فرعية حيث وضعت المجموعة الفرعية الأولى كمجموعة ضابطة موجبة بينما المجموعة الثانية والثالثة تم تغذيتها علي الوجبة الأساسية بالإضافة الي الشاي الاخضر الغير مغلي بتركيز ١.٥ و ٣ مل علي التوالي بالحقن عن طريق الفم. في حين تم تغذية المجموعة الرابعة والخامسة علي الوجبة الأساسية بالإضافة الي الشاي الاخضر المغلي بتركيز ١.٥ و ٣ مل علي التوالي بالحقن عن طريق الفم. وأوضحت النتائج أن تناول كل من الشاي الأخضر المغلي و الغير مغلي أدى الي إنخفاض معنوي في بعض التحاليل مثل معدل إكتساب الوزن - الغذاء المأكول - معدل الإستفادة من الغذاء - التراي جليسرول - الليپروتينات منخفضة الكثافة - الليپروتينات منخفضة الكثافة جدا- ووظائف الكبد (ALT- AST). وعلي الجانب الأخر ادت المعاملات الي ارتفاع معنوي في الليپوبروتينات مرتفع الكثافة وذلك عند مقارنة المجموعة الضابطة الموجبة . ولذا يوصي بأن الشاي الاخضر المغلي والغير مغلي يمكن أن يحسن مستويات دهون الدم و مستوي الأنسولين والجلوكوز ووظائف الكبد .

**الكلمات الافتتاحية :-** الشاي الأخضر- الداء السكري - دهون الدم- ووظائف الكبد - الجلوكوز- الأنسولين.