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Effect of Green Tea Aqueous Extract on Body Weight, Glucose Level, and Kidney Functions in Diabetic Male Albino Rats

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Abstract

The effect of daily oral doses of green tea water extract for 4 weeks on body weight, plasma glucose level, kidney functions in alloxan-induced diabetic rats was investigated. Forty adult male albino rats (150 -160 g) was divided into 4 experimental groups: The first group was considered as control group. The second group was treated with water extract of the green tea (200 mg/kg b.w).The third group was infected by induction of experimental diabetes by intraperitonial injection of (150 mg/kg b.w) alloxan, and the fourth group was infected by induction diabetes and treated with green tea extract. Different physiological parameters were performed including recording of the body weight and measuring blood sugar, creatinine and urea levels. Body weight loses, plasma glucose, creatinine, and urea levels were significantly ($p< 0.05$) reduced in diabetic rats treated with green tea when compared with the diabetic rats. From these results, it can be conclude that the consumption of green tea extract produced a significant reduction in glucose level in diabetic rats. In addition, it is capable of improving the impaired kidney functions in diabetic animals.

Keywords: Green Tea, Alloxan, Diabetic, Kidney Function, Rats.

Introduction

Diabetes mellitus can be defined as a group of metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both resulting in impaired function in carbohydrate, lipid and protein metabolism. There are two forms of diabetes mellitus: type 1 and 2. In type 1 diabetes or, insulin-dependent diabetes mellitus the pancreatic β-cells are progressively destroyed and secrete little or no insulin. Type 2 diabetes or non-insulin-dependent diabetes mellitus, is a heterogeneous disorder of insulin resistance and pancreatic β-cells dysfunction [1]. Hyperglycemia is strongly linked to diabetic complications such as neuropathy, retinopathy and nephropathy. Stringent control of the hyperglycemia by insulin treatment has been shown to avert hypertrophy and hyper filtration and the subsequent rise in urinary protein excretion [2]. Many studies have been focused on traditional and herbal medicines to find novel therapeutic agents for diabetic.

Tea is the most consumed beverage in the world. Green tea is made from the leaves of the warm weather evergreen *Camellia sinensis* L. (Theaceae). The major components of interest are the polyphenols. The major polyphenols in green tea are flavonoids. The four major flavonoids in green tea are the catechins, epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG) and epigallocatechin gallate (EGCG). Epigallocatechin gallate is viewed as the most significant active component. Other compounds of interest in dried green tea leave include gallic acid, quercetin, kaempferol, myricetin, saponins, caffeic acid and chlorogenic acid [3]. Recently green tea is being widely studied for its beneficial effects in the treatment and prevention of human diseases. It has been demonstrated that catechins can reduce blood glucose level [4].

Therefore, it has been expected that an intake of green tea extract will prevent or delay the onset of diseases such as diabetes. Polysaccharides of green tea have also been reported to have hypoglycemic activities [5]. *In vitro* rat studies suggest that EGCG and other catechins and the flavones help prevent hyperglycemia by enhancing insulin activity and possibly by preventing damage to β-cells [6]. Glucose levels in the blood of rats were reduced by EGCG has been shown in rodents to be effective in preventing the development of Type I diabetes and treatment of Type II diabetes [7].Green tea has been reported to

reduce body weight, body mass index and body fat [8]. Clarc *et al.,* reported that diabetic rats treated with green tea showed a significant reduction in creatinine level [9]. GT significantly decreased the blood level, serum creatinine, serum malondialdehyde and kidney excretion of glucose and proteins and oxidative stress in the kidney [10].

The aim of the present study was to investigate the effects of daily oral consumption of green tea extract on body weight, plasma glucose, and kidney functions in alloxan-induced diabetic rats to show the preventive effects of green tea.

Materials and Methods

Animals and experimental design

Forty adult 12 weeks old, male albino rats (*Rattus norvegicus*), weighing 150-160 g were obtained from animal's house of the College of Science, University of Baghdad, Iraq. They were housed in standard plastic cages. The animals were kept in a well ventilated room, temperature of 24- 28°C with 12 hrs natural light and 12 hrs darkness. The rats had free access to tap water and dry rat pellets obtained from local market *ad libitum*. The rats were allowed to acclimatize for ten days and then divided into 4 groups; (10 per group) as follows: untreated control group, green tea group were given GT extract at a dose of 200 mg/kg b.w orally for 4 weeks, diabetic group injected intraperitoneally with (150 mg/kg b.w of alloxan), and diabetic treated with green tea (200 mg/kg b.w) group (the rats first were injected with alloxan then given green tea for 4 weeks).

Preparation of green tea extract and induction of diabetes

Green tea was obtained from a local market and stored in dry atmosphere. Green tea aqueous extract were prepared by dissolving amounts equivalent to 200 mg tea leaf powder per kg body weight in glassware containing 1ml boiling distilled water (equivalent to 6 cups of tea). The solution was kept to stand for10 min before being filtered, cooled to room temperature, and dispensed in clean drinking bottles; the extract was kept at 4°C [11]. Diabetes was induced in fasting rats 12 hrs by a single intraperitoneal injection of 150 mg/kg b.w. of alloxan dissolved in 0·9% saline, and the diabetic state was assessed by measuring the fasting plasma glucose concentration (by enzymatic determination of glucose) 72 hrs after alloxan treatment in fasting rats, and the rats with a plasma glucose level above 250 mg/dl were selected for the experiment and considered as diabetic [12].

Collection of blood samples and biochemical analysis

At the end of the experiment, blood samples were taken by cardiac puncture and blood was collected in clean EDTA tubes, then plasma was separated by centrifugation (3000 rpm for 15 min.) and stored at -20°C. Kits of glucose, creatinine, and urea were used. Kits of glucose, creatinine, and urea were purchased from Spinreact, S.A. Ctra. Santa Coloma, Spain. Glucose determination was carried out according to the method of, [13].Creatinine was determined by kinetic method described by, [14], determination of urea was according to the enzymatic method of [15].

Statistical analysis

Data are expressed as the mean ± SE. The statistical significance was carried out using one- way analysis of variance test followed by Duncan's Multiple Range Test (SPSS statistical software package) [16]. A possibility of *P* value ($p<0.05$) was considered as significant differences between means.

Results and Discussion

Changes in body weight

From Table (1), it is shown that, the average body weight of diabetic rats was significantly ($p< 0.05$) lower than that of the normal control, GT treated group, and diabetic rats treated with GT. Likewise, the body weight of the diabetic rats treated with GT was significantly ($p< 0.05$) lower than the normal control. There was a significant ($p< 0.05$) decrease in the mean body weight of the GT treated rats when compared with normal control groups, and a significant ($p< 0.05$) increase when compared with diabetic groups.

The results revealed that green tea extract decreased body weight gain and had an anti-obesity potential. These findings are in agreement with other investigators. Sayama *et al.,* reported that the addition of green tea powder (GTP) to the diet suppressed fat

accumulation and body weight increase without reduction of food intake in mice [17]. Moreover, it was reported that epigallocatechin gallate (EGCG), a kind of catechin, had an inhibitory effect on acetyl-CoA carboxylase which is essential for *in vitro* fatty acid biosynthesis [18]. Weight loss (one of the clinical features of diabetes) seen in alloxan-treated animals may be due to the degeneration of the adipocytes and muscle tissues to compensate for the energy lost from the body due to frequent urination and over-conversion of glycogen to glucose[19].

The significant decrease in body weight loses induced by green tea extracts in the diabetic rats, suggests that GTE may have a positive anabolic effect by decreasing the degeneration of the adipocytes and muscle tissues through improving glucose metabolism. Based on biochemical and pharmacological studies, the mechanisms of preventing obesity by tea consumption may be through stimulating hepatic lipid metabolism, inhibiting gastric and pancreatic lipases, stimulating thermogenesis and suppressing fatty acid synthase by green tea catechins [20]. Other studies reported that green tea epigallocatechin gallate inhibited lipid absorption through its ability to form complexes with lipids and lipolytic enzymes and hence interfering with the luminal processes of emulsification, hydrolysis, micellar solubilisation, and subsequent uptake of lipids [21].

The effect of green tea on glucose level

Table (1), shows the effect of GT consumption on the levels of plasma glucose. In diabetic animals, the plasma glucose levels were significantly ($p< 0.05$) elevated when compared with normal control. However, diabetic rats treated with GT show a significant ($p<0.05$) reduction in plasma glucose when compared with diabetic groups, but they still significantly higher than the normal rats. In GT treated rats, there was a significant decrease in the plasma glucose when compared with diabetic animals, and there were no significant differences when compared with normal control.

Treatment of diabetic animals with green tea did not decrease blood glucose levels to the normal, however is still significantly higher than normal, these results are in agreement with several studies. It has been reported that green tea reduced blood glucose level in both type 1 and type 2 of diabetic rat's models [22, 23]. Rupasinghe *etal.,* reported that saponins, flavonoids, phenolic compounds and glycosides have hypoglycemic effects [24]. Several studies reported that the hypoglycemic effect of green tea was attributed to the presence of polyphenols, catechins and a water-soluble polysaccharide fraction [25, 26]. It has been reported that green tea polyphenols increase insulin activity in diabetic rats [6]. It also is documented that the water-soluble polysaccharides fraction of green tea is responsible for its anitdiabetic effect [27].

It was also found that tea consumption significantly decreased blood glucose levels by increasing hepatic glycogen level in alloxan-diabetic rats possibly through reactivation of the glycogen synthase system (as a result of increased insulin secretion) and decreasing liver glucose-6-phosphatase activity, which is mainly responsible for releasing glucose molecules to the blood by converting glucose-6-phosphate to glucose [25,28]. Some studies have shown that catechins can inhibit digestive enzymes such as salivary amylase, intestinal sucrase and α-glucosidase, suggesting that the reduced digestibility action of catechins may be responsible for lowering blood glucose levels in diabetic rats, and these mechanisms may be responsible for the anti-hyperglycemic effect of green tea [29]. Other studies reported that green tea epigallocatechin gallate promotes pancreatic β-cells regeneration in alloxan-treated rats, has insulin-like and insulinotropic activities, and inhibits gluconeogenesis through inhibition of liver phosphoenolpyruvate kinase synthesis [30].

The effect of green tea on the creatinine and urea levels

Table (1), also shows the effects of GT treatment on plasma urea and creatinine under conditions of diabetes. The plasma urea and creatinine level were significantly ($p< 0.05$) higher in diabetic animals than control rats, whereas the administration of green tea extract to diabetic rats recorded a significant reduction in the levels of plasma urea and creatinine when compared with diabetic rats, but they still significantly higher than the normal rats.

While, no significant differences were observed in the levels of plasma creatinine and urea in green tea treated rats when compared with normal control, but they still significantly lower than the diabetic rats.

Alloxan-induced diabetes caused increasing plasma levels of creatinine, and urea, and also alloxan produces oxygen radicals and oxidative stress in the body, [31]. Another interpretation made by [19], reported that green tea catechin has been found to improve the percentage of prostaglandin.The catechin enables the kidney malfunctions resulting from diabetes to return to normal state.

Plasma creatinine and blood urea levels were found to be reduced by the administration of green tea polyphenols in alloxan-induced diabetic rats, also the GT reduced serum glucose and creatinine levels and serum lipid peroxidation and increased serum superoxide dismutase, suggesting that catechins influence glucose metabolism and improve kidney function by reducing oxidative stress in alloxan-treated diabetic rats [25], and this may interpreted the results obtained in this study. It was concluded from the current study that the taken doses of green tea extract are affected positively in reducing glucose level and improving the impaired kidney functions in diabetic rats.

Table (1)
Body weight and levels of plasma glucose, creatinine, and urea in alloxan–induced diabetic rats received 200 mg/kg b.w. green tea aqueous extract.

Groups → / Parameters ↓	*Normal control group*	*Green tea group(treated with green tea 200 mg/kg b.w.)*	*Diabetic group (treated with alloxan 150 mg/kg b.w.)*	*Diabetic group treated with green tea 200 mg/kg b.w.*
Initial body weight. g	157.4 ± 1.24	153.8 ± 1.74	155.6 ± 2.03	154.4 ± 1.16
Final body weight. g	181.4 ± 0.67	175 ± 2*#	142.4 ± 0.67*	162.6 ± 2.78*#
Glucose mg /dl	112.6 ± 1.83	106.8 ± 1.46 #	321.6 ± 8.4*	182.6 ± 3.3 *#
Creatinine mg /dl	0.468 ± 0.01	0.64 ± 0.05 #	3.14 ± 0.18*	1.42 ± 0.13*#
Urea mg /dl	32 ± 1.14	35.4 ± 2.31 #	71.2 ± 1.88*	58.2 ± 1.56*#

Values are expressed as mean ± S.E of 10 animals.
**** Values are statistically significant P <0.05 when compared with normal control.***
 # Values are statistically significant P <0.05 when compared with diabetic group.

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الخلاصة

تمت دراسة تأثير التناول اليومي للمستخلص المائي للشاي الاخضر لمدة اربعة اسابيع في وزن الجسم ومستوى الكلوكوزووظائف الكلى، في ذكور الجرذان البيض المصابة بالسكري المستحث بالالوكسان.استعملت اربعون جرذا من ذكور الجرذان البيض البالغة تراوحت اوزانها ما بين ١٥٠ الى ١٦٠ غرام قسمت الى اربع مجاميع: جرعت المجموعة الاولى كمجموعة سيطرة وتلقت المجموعة الثانية جرعة من الالوكسان مقدارها ١٥٠ ملغرام/ لكل كيلوغرام من وزن الجسم عن طريق الحقن بالبريتون. وجرعت المجموعة الثالثة بمستخلص مائي من الشاي الاخضر بجرعة مقدارها ٢٠٠ ملغرام / لكل كيلوغرام من وزن الجسم يوميا وعن طريق الفم لمدة ٤ اسابيع والمجموعة الرابعة جرعت بالالوكسان ثم جرعت بمستخلص الشاي الاخضر وكما في المجموعتين الثانية والثالثة. تم قياس عدة معايير فسلجية تضمنت وزن الجسم ومستوى السكر ومستوى اليوريا والكرياتنين. اظهرت النتائج وجود انخفاض معنوي في معدل فقدان وزن الجسم ومستويات السكر واليوريا والكرياتنين في دم الجرذان المصابة بالسكري والمعاملة بمستخلص الشاي الاخضر عند المقارنة بالجرذان المصابة بالسكري. ومن نتائج الدراسة يتضح ان المعاملة بمستخلص الشاي الاخضر لها تاثير واضح في خفض مستوى السكر في الجرذان المصابة بالسكري، فضلا عن قدرته على تعديل الخلل في وظائف الكلى.