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Effect of the Aqueous Green Leaf Extract of Green Tea (*Camellia sinensis*) on Glucose Level of Rat

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Abstract: *Camellia sinensis* or tea belonging to the family of Theaceae, is widely grown. In Mazandaran provinces in the north of Iran. Tea is known in folk medicine as a medicinal plant that used as hypotensive and anti-diabetic. In this research the aqueous green leaf extract of *Camellia sinensis* (450 mg kg⁻¹) showed a strong glucose lowering effect after oral administration in rats. The decrease of glycemia has reached to 30% of the control value 2 h after glucose loading. The amount of glucose absorbed in a segment jejunum in situ was 9.2±0.2 mg in presence of tea extract vs. 14.11±0.91 mg in control rats during 2 h (p<0.05). The results indicate that aqueous extract of tea has a significant anti hyperglycemic effect that may be caused in part by the reduction of intestinal glucose absorption.

Key words: *Camellia sinensis*, green leaf, anti-hyperglycemia, Iran

INTRODUCTION

Many indigenous Iranian medicinal plants have been found to be useful to successfully manage diabetes (Shokrzadeh *et al.*, 2005; Ebadi *et al.*, 2005). Despite the introduction of hypoglycemic agents from natural and synthetic sources diabetes and its complications continue to be a major problem in the world population.

Green tea (leaves of *Camellia sinensis*, Theaceae) is a popular beverage in East Asia and also used as a herbal remedy in Europe and North America. Green tea is considered to be antiinflammatory, antioxidative, antimutagenic and anticarcinogenic (Aebi, 1974; Bergmeyer and Bernt, 1980; Beutler *et al.*, 1963; Choi *et al.*, 1998; Cooperstien and Walkins, 1981) and can prevent cardiac disorders. Epidemiologically, it has been suggested that green tea consumption prevents type 2 diabetes. The amelioration of insulin resistance by green tea is associated with the increased expression level of glucose transporter IV in a fructose-fed rat (Wu *et al.*, 2004). Green tea extract contains polyphenols (e.g., catechin, epicatechin, epigallocatechin and their gallates), teanin and caffeine. Some constituent components have been shown to enhance the basal and insulin-stimulated glucose uptake of rat adipocytes (Wu *et al.*, 2004), to inhibit intestinal glucose uptake by inhibiting the sodium-dependent glucose transporter of rabbit intestinal epithelial cells (Kobayashi *et al.*, 2000). Controversially, caffeine acutely lowers insulin sensitivity in humans

(Ebadi *et al.*, 2005; Kunchandy and Rao, 1990; Gomes *et al.*, 1995; Halliwell and Gutteridge, 1985; Hara, 1994; Hassid and Abraham, 1957; Helen and Vijayammal, 1997; Illing *et al.*, 1951; Joy and Kuttan, 1999; Kaszkin *et al.*, 2004; King and Armstrong, 1980).

The purpose of this study is to study the hypoglycemic effect of aqueous leaf extract of *C. sinensis* grown in Mazandarn province on hyperglycemia induced by Oral Glucose Tolerance Test (OGTT) and on alloxan-induced diabetic rats. The intestinal glucose absorption was measured in situ in a perfused jejunum segment in order to determine one of the probable mechanism of tea's antihyperglycemic effect.

MATERIALS AND METHODS

All procedures in this study was carried out at December 2003, in Central laboratory of Mazandaran medical canter (Sari-Iran). Tea was collected from some areas of Mazandaran province (Ramsar city) in the north of Iran and prepared for experiment based on Wu *et al.* (2004). Male Wistar rats weighing 250-350 g prepared from Pasteur institute and were kept in a room maintained at a temperature of 23°C. Animals were fasted for 16 h before the OGTT. Glucose (1 g kg⁻¹) was administered by gavages 30 min after oral administration of 450 mg kg⁻¹ of *C. sinensis* leaf water extract. Glibenclamide at dose of 2 mg kg⁻¹ was used as a reference drug. Blood glucose level was measured each hour after glucose loading in rats

under light ether anesthesia. Rats were treated intraperitoneally with 150 mg kg⁻¹ day⁻¹ of alloxan for 3 consecutive days. Three days after the last alloxan injection, only rats with fasting glycemia more than 1.5 g L⁻¹ were used. They were divided into two groups and after 16 h of fasting, treated orally with 450 mg kg⁻¹ of tea extract or distilled Water (control), respectively. Blood samples were obtained 30 min before and 60, 120, 180 min after treatment. The effect of the water leaf extract obtained from tea was tested in a perfused Jejunum segment (6 cm) in fasted rats for 36 h and anaesthetized with sodium pentobarbital (50 mg kg⁻¹, i.m.). The bilberry infusion was added to a 3 final concentration 450 mg kg⁻¹. The system was set at constant temperature of 37°C and the perfusion rate was 0.53 mL min⁻¹ for 2 h. The controls were perfused with the perfusing solution without tea extract. Student's t-test was used for statistical analysis and p<0.05 was considered to be significant (Bergmeyer and Bernt, 1980).

RESULTS AND DISCUSSION

A strong antihyperglycemic effect of *C. sinensis* (450 mg kg⁻¹) was observed at the first Hour after glucose loading in rats under OGTT (Table 1). In alloxan-induced diabetic rats, the oral administration of the aqueous extract of *C. sinensis* did not modify the blood glucose level. On the contrary, a strong antihyperglycemic effect of *C. sinensis* (450 mg kg⁻¹) was observed. The fall of glycemia was approximately 30% vs. control. This effect was still present 120 min after the oral administration of glucose.

Glibenclamide at dose of 2 mg kg⁻¹ resulted more active at all the time tested. The intestinal glucose absorption in situ on jejunum segment showed that leaf water extract significantly reduced the absorption of glucose. The amount of glucose absorbed in controls during 2 h was (13.88±0.91 mg) vs. (10.2±0.32 mg). In the presence of bilberry extract (p<0.05).

We have not observed any sign of intestinal irritation due to the presence of tea aqueous extract. Tea traditionally has been used for a number of ailments including diabetes. In the present study we demonstrated an antihyperglycemic effect of the aqueous leaf extract of *C. sinensis* grown in Mazandaran province. The lack of hypoglycemic effect of tea aqueous leaf extract in alloxan-induced diabetic rats which is a model of diabetes with hypoinsulinemia demonstrates that this extract may act on glucose homeostasis by extrapancreatic way. It indicates that the presence of insulin is required for the hypoglycemic activity of tea. We have shown that bilberry inhibits significantly glucose absorption in small

Table 1: Effect of oral treatment of 450 mg kg⁻¹ of *C. sinensis* aqueous leaf extract on plasma glucose level of rats. Values are means

Glibenclamide	Glucose level (g L ⁻¹)		
	Time (min)	Controls	Leaf extract
1.13	-30	1.20	1.31
1.13	0	1.28	1.20
1.13	30	1.30	0.95
1.13	60	1.40	0.82
0.83	90	1.31	0.87
0.83	120	1.30	0.87
0.83	150	1.29	0.85
0.84	180	1.29	0.85

intestine in rats under anesthesia. This can be considered as one of the mechanisms by which this extract can regulate the glucose homeostasis in glucose loaded rats. However, this result does not exclude the other mechanisms regulating the peripheral glucose homeostasis.

Green tea is widely consumed in Asian countries, while black tea is most popular in Western countries. The manufacturing process of green tea differs from that of black tea because freshly picked young leaves of the tea are immediately steamed. This process destroys the enzymes responsible for breaking down the color pigments in the leaves and allows the tea to maintain its green color during the subsequent rolling and drying processes. The amounts of constituent compounds are slightly different from those of black tea. Pharmacological studies using constituent compounds in green tea have been recently reviewed by Kaszkin *et al.* (2004). Green tea extracts are more stable than pure epigallocatechin gallate, the major constituents of green tea, because of the presence of other antioxidant constituents in the extract (Kaszkin *et al.*, 2004). In general, herbal medicines are complex mixtures of different compounds that often act in a synergistic fashion and exert their full beneficial effect as total extracts (Loew and Kaszkin, 2002; Kobayashi *et al.*, 2000; Loew and Kaszkin, 2002; Lowry *et al.*, 1951; McCord and Fridovich, 1969; Masayasu and Hiroshi, 1979).

In conclusion, the results indicate that aqueous extract of *C. sinensis* grown in Mazandaran province had a significant antihyperglycemic effect which may be caused in part by the reduction of intestinal glucose absorption. The integration of a menu with bilberry may be possible and recommendable for the management of diabetes. Other experiments are necessary to determine the other mechanisms of antihyperglycemic action of bilberry and the active fractions involved in this effect. However, in some studies of laboratory cultures and animals, an extract of tea leaves has shown the opposite effect but in many cases, researchers noted that animals treated with bilberry water leaf extract produced increased

amounts of insulin. In one of the studies, decreased blood sugar occurred in animals with both high and normal blood sugar levels (Mukhtar *et al.*, 1992; Nagarajan *et al.*, 1987; Ohkawa *et al.*, 1979; Perfumi and Tacconi, 1996; Ponnachan *et al.*, 1993; Rajasekharan and Tuli, 1976; Sano *et al.*, 1995). We observed that green tea improved oral glucose tolerance in rats. It is therefore likely that green tea is prophylactic against diabetes and ameliorates diabetic hyperglycemia. Green tea consumption at moderate doses may be associated with a reduced risk of type 2 diabetes in apparently healthy individuals by controlling postprandial hyperglycemia. Much more research is needed to prove or disprove the possible effects of tea's aerial parts on blood sugar (Sato, 1978; Shokrzadeh *et al.*, 2005; Subramoniam *et al.*, 1996; Shim *et al.*, 1995; Vucic *et al.*, 1997; Yang *et al.*, 1999).

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