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REGULAR ARTICLE

Anti-diabetic effect of *Coffea arabica*, in alloxan-induced diabetic rats

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Abstract

The coffee bean could provide benefits in cardiovascular and metabolic diseases. The anti-diabetic effect of extracts of *Coffea arabica* was evaluated in diabetic rats. The aqueous extract of coffee green grain (63 and 93mg/kg) was administered once daily for fifteen days to alloxan-induced diabetic rats. The effect of aqueous extract on fasting blood glucose levels was measured. After 8 and 15 day of treatment, aqueous extract of coffee green grain administration showed significantly lower blood glucose levels compared to the diabetic control group. The findings from this study suggest that extract of coffee can alleviate hyperglycemia of diabetes.

Key words: Anti-diabetic effect, Aqueous extract, *Coffea arabica*

Introduction

Coffee is the most popular beverage in the world after water (George et al., 2008). It is obtained from the processing of the fruits of coffee tree, whole plant of the genus Coffea, Rubiaceae family (Davies et al., 2006; Castilla, 2012). They grow in more than 80 countries in tropical and subtropical regions, especially in Africa, Asia and Latin America (Mishra et al., 2012).

A great number of substances were found in coffee beans, green or roasted and have been studied for many years, such as aliphatic and aromatic compounds, among which are alcohols, aldehydes, carboxylic acids and esters, heterocyclic compounds, proteins, amino acids and nucleic acids, carbohydrates, lipids, like sterols, tocopherols and diterpenes, alkaloids such as caffeine, theobromine, theophylline, trigonelline, adenine, guanine, hypoxanthine and xanthine; also micronutrients such as magnesium and potassium (Spiller, 1998). Certainly caffeine is best known, however cafestol, kahweol and chlorogenic acid, are also compounds present in coffee with antioxidant properties attributed (Higdon et al., 2006). The total percentage of chlorogenic acid varies by state found in the coffee bean. Some authors reported that the chlorogenic acid in green beans of *Coffea arabica* is 5.5 to 8.0% and in the roasted bean is 1.2 to 2.3% (Bolivar et al., 2009).

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from the altered insulin secretion, insulin action, or both (Norris et al., 2009). Chronic hyperglycemia of DM is associated with long-term damage, dysfunction and organ failure as particularly eyes, kidneys, nerves, heart and blood vessels (Guzmán et al., 2010). Sustained hyperglycemia leads to oxidative stress, alterations in enzyme activity, protein glycosylation and several structural changes (Akpan et al., 2007).

Epidemiological studies conducted in recent years (van Dam and Feskens, 2002; Isogawa et al., 2003; Salazar-Martinez, 2004) revealed an inverse association between coffee consumption and the prevalence of DM2. There are also data from animal studies indicating that caffeine (Shi, 1997), but most of chlorogenic acid and derivatives (Arion et al., 1997; Clifford, 2000; Ong et al., 2012; Hunyadi et al., 2012) have hypoglycemic effect by different mechanisms. Likewise, magnesium administration has been associated with reduction of hyperglycemia induced by alloxan (Abayomi et al., 2011).

The alloxan has been commonly used as an agent to induce experimental diabetes model; exerts

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its action when administered parenterally, generates reactive oxygen species (ROS) and elevated cytosolic calcium in pancreatic islet B (Szkudelski, 2001).

Vegetables are an important source of antioxidants; high levels of these compounds in the diet is believed to help reduce certain diseases (Astley, 2003; Bazzano et al., 2002), even more if ROS are involved in the pathogenesis (Akpan et al., 2007).

This study evaluated the effect of the aqueous extract of *Coffea arabica* on hyperglycemia level in rats with alloxan-induced diabetic.

Materials and Methods

Plant material

Samples were collected in Jazan (Pedro Ruiz Gallo, Bongará district, province of Amazonas Region). The identification was made by Dr. Isidoro Sánchez Vega from The Herbarium of the National University of Cajamarca and Research Associate of The Field Museum, Chicago, Illinois, USA.

Animals

Holtzman male rats (200-230g) were purchased from the National Institute of Health (INS-Lima, Peru). Before starting the experiments, the animals were acclimatized for 10 days. The rats were maintained on a 12 hour light / dark at 22 ± 2°C. They received a diet of standardized pellets and water *ad libitum*. In the use of animals for experimentation we have followed the guidelines of the Institutional Committee of Ethic for the Use of Animals from the Universidad Peruana Cayetano Heredia de Lima, Peru (UPCH), with whom the Universidad Privada Antonio Guillermo Urrelo, Cajamarca, Peru -UPAGU) has an interinstitutional signed agreement.

Chemicals and reagents

Alloxan monohydrate was purchased from Sigma-Aldrich (St Louis, MO, USA), glucose kit from Wiener Lab (Rosario, Argentina), glyburide was from Farmindustria Lab (Lima, Peru) and sodium hypochlorite from Peruvian Merck Laboratories (Lima, Peru).

Preparation of extracts

After harvesting, the green beans were selected from *Coffea arabica* L. "Coffee", washed with water and sodium hypochlorite 1% (v/v), to subsequently carry out the stability process at a temperature of 120°C for 5 minutes. The aqueous extract was made using a decoction, for which weighed 15 g of coffee green beans and added 100 mL of distilled water in order to boil for 15 minutes. Then we proceeded to filter through sterile gauze. The extract was poured into glass jar amber. The dose was obtained on dry matter.

Induction of Diabetes mellitus

Diabetes was induced by a single intraperitoneal administration of alloxan (140mg/kg) with 4% saline solution (an average of 0.90 mL per specimen). Before administering alloxan, we took a baseline glycemia. After twelve hours were extracted from blood samples by puncture of the tail vein. After five days the rats showed levels above 300mg/dL were considered diabetic. One group of rats not administered alloxan and served as normal control. Glyburide (0.36mg/kg) was used as a reference point.

Experimental design

The animals were randomized into 5 groups, consisting of six specimens each group:

Treatments were administered orally for 15 days once a day from the eighth day of induction of diabetes. The distribution of the specimens was as follows:

- Group I: Non-diabetic rats received saline solution (2mL/kg) served as normal control group.
- Group II: Diabetic rats received saline solution (2mL/kg) served as diabetic control group.
- Group III: Diabetic rats received aqueous extract of *Coffea arabica* grain at a dose of 63mg/kg.
- Group IV: Diabetic rats received aqueous extract of *Coffea arabica* grain at a dose of 93mg/kg.
- Group V: Diabetic rats received a dose of glyburide 0.36mg/kg serves as a control reference.

Determination of glycemia

After an overnight fast (12 -14 hours), we took blood samples (once a week) and the serum was obtained by centrifugation (1,096 x g). To determine the blood glucose we proceeded by the glucose oxidase method. The results are reported in mg/dL.

Statistical analysis

For analysis of statistical comparisons the program SPSS v. 19.0 (Statistical Package for Social Sciences) were used. We determined the mean and standard deviation for quantitative variables making a comparison between post-treatment values of the groups through ANOVA and Student t test, with a significance level of $p < 0.05$.

Results and Discussion

The diabetes health significance due to cardiovascular morbidity, based on a condition where there is a manifest of plasma glucose levels higher than normal. The consensus is clear about that intensive treatment of all factors risk are the best way to prevent or delay diabetes-associated morbidity, that is why reducing hyperglycemia to prevent the occurrence of these effects is the best strategy hypoglycemic activity (Jara, 2006).

Plasma levels baseline of glucose are observed in the study groups (Table 1), ranging from 105 to 115 mg/dL. In normal rats, with free access to food and water, glucose values between 70 and 135mg/dL (Nitz et al., 2003), although variations may be higher, depending on the strain and the type of food they receive. This shows that our specimens are homogeneous and comparable in status and energy metabolism, and ensures that our results are more reliable.

Although a pharmacological model cannot be extrapolated to a complex human pathology, the use of alloxan is accepted as a chemical induction model resembles human diabetes (González, 2006). After receiving alloxan to the single dose of 140mg/kg, the glucose levels in groups II,III, IV and V (Table 1) showed a significant increase ($p < 0.05$) and significantly higher, an increase that exceeds 400% of the values basal glucose.

The alloxan can be administered intravenously at a dose of 40 to 45mg/kg or intraperitoneally between 50 and 200mg/kg. When using the higher dose the glucose levels rise to levels ranging from 150 to 900mg/kg, however the percentage of diabetic rats obtained is variable and depends on the sensitivity of the rats (Di Loreto, 2003).

Alloxan response can be divided into three phases. The initial hyperglycemia lasting about two hours, probably due to hepatic glycogenolysis; transient hypoglycemia, at approximately 6 hours, due to the output of insulin from damaged cells and finally hyperglycemia permanent begins at 12 hours. This diabetogenic agent forming free radicals, depletion of NAD^+ due to DNA damage and inhibition of glucosinase within mitochondrial changes include decreased reduced glutathione, calcium and deficient ATP (Szkudelski, 2001).

After eight days of treatment with the aqueous extract of *Coffea arabica* L. in groups III and IV (Table 2) at doses of 62 and 93mg/kg respectively, we observed a statistically significant reduction ($p < 0.05$) in group IV and group V compared to day "0". Similarly if we compare intergroups, we note that the group III had no significant effect compared with groups IV and V. The higher dose of *Coffea arabica* reduces glucose levels in 71%, while glyburide reduces that in 56%.

Table 1. Blood glucose before and after administra tion of diabetogenic agent (alloxan) in the study groups.

Glycemia (mg/dL)	Group I	Group II	Group III	Group IV	Group V
Day -4 (baseline)	108.17±24.03	111.58±17.71	105.67±19.40	115.83±25.57	112.83±17.71
Day 0 (alloxan)	106.55±22.09	583.25±22.84$^{\#}$	600.33±1.51$^{\#}$ (+468%)	600.33±2.66$^{\#}$ (+418%)	570.00±46.90$^{\#}$ (+405%)

n = 6

Groups II, III, IV and V are alloxan.

p <0, 05 compared with baseline glycemia.

Table 2. Blood glucose in alloxan post "day 0", day 8 and day 15 in the study groups.

Glycemia (mg/dL)	Group I	Group II	Group III	Group IV	Group V
Day 0	106.55±22.09	583.25±22.84	600.33±1.51	600.33±2.66	570.00±46.90
Day 8	105.23±18.44	492.54±32.56$^{\#}$ (-16%)	513.67±103.38$^{\#}$ (-15%)	172.67±36.92$^{+,*}$ (-71%)	248.67±46.68$^{\#,+,*}$ (-56%)
Day 15	108.16±20.32	476.65±38.52$^{\#}$ (-18%)	93.33±7.45$^{+,*,\&}$ (-83%)	97.50±7.59$^{+,*,\&}$ (-84%)	148.50±28.20$^{+,*}$ (-74%)

Drugs test material begins to manage when the animals are experimentally diabetic. The groups III and IV are 62 and 93mg dose / kg of aqueous extract of coffee, respectively; glyburide group V received a dose of 0.36mg/kg.

Intergroup:

# p <0.05 compared with group I

+ p <0.05 compared with group II

intragroup:

*p <0.05 compared with day 0

&p <0.05 compared with day 8

Glyburide is part of the standard treatment of Type 2 Diabetes mellitus. Like other members of this pharmacological group, the channels block ATP-dependent potassium found in the membranes of type β pancreatic cells, causing depolarization, calcium entry and insulin release. Also decreases hepatic glycogenolysis and gluconeogenesis (Alquilante, 2010; Schelleman, 2010).

After completing the fifteen day treatment, we observed (Table 2) a reduction of over 80% at two tested doses of *Coffea arabica*, while glyburide achieves a 74% reduction. The effect of coffee extract is more intense than the sulfonylurea, showing that the longer the administration hypoglycemic effect is better.

Coffee is a complex beverage with hundreds of components present in the grain or produced in the process and in the development of the beverage. Thus, there has been identified more than 700 volatile compounds from several categories in roasted coffee beans, as well as numerous nonvolatile components such as polysaccharides, melanoidins, chlorogenic acids, aldehydes, ketones, alkaloids such as caffeine and inorganic compounds such as nitrogen, potassium, calcium, magnesium, phosphorus and sulfur. To this is added the compounds formed during processing of the beverage (Gil et al., 2004).

Under our findings, we must focus our discussion on active components that justifies the effect found. However, from a purely pharmacological point of view, it is difficult to attribute the effect only to a component or a small group of them, especially if these effects are fuzzy.

Crist et al. (1998) found that caffeine administered to obese rats inhibits glucose uptake in adipose tissue. Wachmann et al. (1970) over forty years ago showed that coffee intake in healthy volunteers, increased glycemia after glucose tolerance test. Ten years ago Keijzers et al. (2012) showed, also in non-diabetic volunteers that caffeine intake reduces sensitivity to insulin, which explain the effect of increased plasma levels of adrenaline and free fatty acids causing consumption of caffeine. Greer et al. (2001) found caffeine reducing effect on glucose uptake; Thong et al. (2002) also found similar effects.

The methodological difficulty of all these authors is that they measure blood glucose levels after acute intake of coffee and / or caffeine. As we know drug intake leads to prolonged adaptation effects; this leads us to speak of tolerance to caffeine, as Naismith et al. demonstrated. In 1970, it showed that in nondiabetic volunteers coffee consumption over a period of 14 days caused a reduction in plasma levels of fasting glucose. Shi (1997) evaluated the effect of caffeine found in animal models serving as stimulating insulin secretion.

As previously mentioned, the effects of coffee and caffeine are especially diffuse. However, epidemiological studies carried out in a large number of subjects, support the use of coffee for diabetes prevention. For example Isogawa et al. (2003) with a sample of 4620 adult subjects revealed that coffee consumption was inversely associated with the prevalence of fasting hyperglycemia.

The path must not necessarily focus only on caffeine, although there is positive evidence. Clifford (2000) reported that the chlorogenic acid present in the coffee beverage, reduces intestinal absorption of glucose and cellular oxidative stress, the reduction of glucose uptake is due to the inhibition of glucose-6-phosphate translocase and reduced sodium gradient driven apical glucose transport (McCarty, 2005). It has also been reported to inhibit the hydrolysis of glucose-6-phosphate to inhibit glucose-6-phosphatase, which might reduce the release of glucose by liver (Arion, 1997). The presence of magnesium is also important. Abayomi et al. (2011) reported that magnesium improves tissue sensitivity and insulin secretion.

Our findings, therefore, are justified by the presence of chlorogenic acid, magnesium and even caffeine. Since in our study the administration has been done for two weeks, it supports the hypothesis of glucose tolerance and other previously mentioned studies. It has been demonstrated that chlorogenic acid on one hand act as a trophic factor and protect pancreatic beta cells and moreover decrease intestinal absorption of glucose, increasing the levels of glucagon-like peptide-1 (GLP-1) and decreasing insulinotropic polypeptide both glucose-dependent (GIP), phenomena that result in a lower glycemic index. The quinolactonas or quinidas also present in coffee which in turn increase the uptake of glucose by peripheral tissues (Johnston et al., 2003).

However, it is necessary to carry experimental studies to evaluate the effect of coffee on lipid levels, as there is evidence (Urgert et al., 1997) that two compounds found in drinking coffee, cafestol and kahweol, could increase blood lipids, although their presence depends on the mode of obtaining the extract.

Conclusions

This study reveals that the aqueous extract of *Coffea arabica* reduces blood glucose levels and thus helps to know the effect of coffee on diabetes mellitus.

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