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ORIGINAL ARTICLE

In vitro and *In vivo* Postprandial Glycemic Activity of *Citrus limetta* Peel Flour

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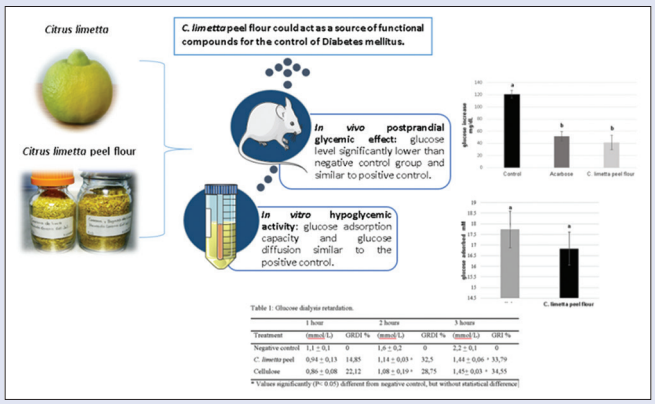
ABSTRACT

Background: Previous studies of *Citrus* spp. peel have shown hypoglycemic and antioxidant activities. *Citrus limetta* has been studied for its therapeutic properties. Diabetes mellitus (DM) is a health problem in Mexico and worldwide, that takes a vital importance due to its high incidence. Recently, scientists have searched natural sources to control the disease. **Materials and Methods:** In this study, we evaluated the *in vitro* hypoglycemic activity and *in vivo* postprandial glycemic effect of *C. limetta* peel flour by glucose adsorption and retardation assays as well as postprandial serum glucose levels using a group of female Balb-c mice, respectively. **Results:** *C. limetta* peel flour showed a glucose adsorption capacity of 16.58 mM, having a similar effect regarding the positive control. The glucose diffusion in the dialysate was elevated, with a glucose dialysis retardation index of 33.79% in a period of 3 h, showing similar results to positive control. Postprandial serum glucose levels in the animal group treated with *C. limetta* peel flour showed a glucose level of 41.4 mg/dL, being this value significantly lower than negative control group and similar to positive control. Toxicity tests showed good tolerance to the dose of 2000 mg/kg. **Conclusion:** *C. limetta* peel flour could act as a source of functional compounds for the control of DM.

Key words: *Citrus limetta*, diabetes mellitus, hypoglycemic, peel flour

SUMMARY

- *Citrus limetta* peel flour showed a glucose adsorption capacity similar to the positive control
- The glucose diffusion in the dialysate was elevated, showing similar results to positive control
- Postprandial serum glucose levels in the animal group treated with *C. limetta* peel flour showed a glucose level significantly lower than negative control group and similar to positive control
- Toxicity tests showed good tolerance
- *C. limetta* peel flour could act as a source of functional compounds for the control of diabetes mellitus.



Abbreviations used: CIATEJ: Center for Research and Assistance in Technology and Design of Jalisco; DM: Diabetes mellitus; FGC: Final glucose concentration; GDRI: Glucose dialysis retardation index; IGC: Initial glucose concentration; OECD: Organization for Economic Cooperation and Development.

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INTRODUCTION

Diabetes is a metabolic disorder caused by pancreas incapacity for insulin production in sufficient quantities resulting in hyperglycemia in the organisms, which may result in other serious effects.[1] Diverse pharmacological approaches are used to diabetes treatment through different action modes, such as the carbohydrate enzyme inhibitors.[2] However, they are responsible for a large number of side effects, making them less attractive as therapeutic agents and placing to the natural remedies as viable alternative. High glucose levels can be effectively controlled through the use of natural products, presenting side effects less frequently and implies a low-cost alternative.[3-6]

Among the products that have shown hypoglycemic activity are citrus peels from orange, grapefruit, lemon, and others. They have been used as traditional medicine in rural communities for prevention and treatment of diabetes.[7] *Citrus limetta*, an edible fruit from Central America known as sweet lime used for human consumption, is comprised 8%–10% peel, in case of juice industries; the peel is a byproduct without any use, becoming an environmental problem.[8] However, the *C. limetta* peel contains physiological beneficial metabolites such as glucosides, flavonoids, and abscisic acid derivatives.[9,10] We previously reported the ability of the *C. limetta* peel to inhibit α-glucosidase and α-amylase enzymes responsible for carbohydrate digestion, which could be exploited for use as an alternative for the control of hyperglycemia

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in people with type 2 diabetes mellitus (DM).[11] In the present study, *in vitro* hypoglycemic activity and *in vivo* postprandial glycemic effect and acute toxicity study of *C. limetta* peel flour were determined.

MATERIALS AND METHODS

Plant material

C. limetta fruits were obtained from a market in Jalisco, Mexico. The pulp was discarded and the peel was dried at 37°C, and then, it was ground and sieved in 50 mesh. The *C. limetta* peel flour was packaged until its use.

Experimental animals

Eight-week-old female Balb-c mice (~22 g body weight) were purchased from the University of Guadalajara, Mexico. They were housed under environmental conditions according to national normativity for the care of laboratory animals. This study was conducted in accordance with the National Institute of Health's "Guide for the Care and Use of Laboratory Animals," and the animals were handled following the animal care guidelines in accordance with regulations enacted by the Federal Government of Mexico (NOM-062-ZOO-1999). An internal committee of the Center for Research and Assistance in Technology and Design of Jalisco reviewed the protocol for the care of laboratory animals.

Glucose adsorption capacity

One percent of *C. limetta* peel flour was added to 25 mL of glucose 100 mM/L in triplicate, according to Ahmed *et al.*[11] The mixture was incubated in an orbital rotatory shaker at 37°C for 6 h. Then, it was centrifuged at 4000 ×*g* for 20 min. Finally, the amount of glucose adsorbed by *C. limetta* flour was measured in the supernatant using a commercial enzymatic kit (GodPap, Randox). Cellulose was utilized as positive control. Glucose bound was calculated as following:

$$\text{Glucose bound} = \frac{\text{IGC} - \text{FGC}}{\text{Weight of the sample}} \times \text{Solution volume}$$

Where IGC is the initial glucose concentration of the original solution and FGC is the final glucose concentration after 6 h of incubation.

Glucose dialysis retardation index

The glucose dialysis retardation index (GDRI) was carried out according to Ou *et al.*[12] with some modifications. Briefly, 0.5 g of *C. limetta* peel flour was mixed in a 100 mM glucose solution (25 mL), and then, it was dialyzed in dialysis bags (12,000 MW cutoff) against 200 mL of distilled water at 37°C during 3 h. The glucose content in the dialysate was measured each hour with a commercial enzymatic kit (GodPap, Randox). Cellulose was used as positive control. The GRDI was determined as follows:

$$\text{GRDI} = 100 - \frac{\text{Total glucose diffused from } \textit{Citrus limetta} \text{ peel flour or cellulose}}{\text{Total glucose diffused from control sample}} \times 100$$

Postprandial hypoglycemic activity

Female Balb-c mice were randomly assigned to three groups of five mice in each. Blood samples were collected from tail veins for glucose levels measurement at baseline on each group after 8 h fasting. The first group (control) was orally administrated purified water (10 ml/kg), the second group was orally administrated 300 mg/kg of *C. limetta* peel flour diluted in purified water, and the third group was administrated the reference drug, acarbose (30 mg/kg). Then, the three groups were induced with hyperglycemia by oral administration of maltose (3 g/kg body weight). Test sample and controls were administered by oral gavage. Blood samples were collected from the mice tail vein before and 30 min after maltose administration, and glucose levels were measured based on the glucose oxidase method using a glucometer (ACCU-CHEK Meter®, Roche Diagnostics Corp., USA).[13]

Acute toxicity study

Acute toxicity was realized according to up-and-down method Organization for Economic Cooperation and Development[14] in five female mice. They were orally administrated with a single dose of 2000 mg/kg (limit test) of *C. limetta* peel flour. All physical and behavioral changes and death were recorded during 14 days.

Statistical analysis

All data were expressed as mean ± standard deviation on triplicate. The data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons using Statgraphics 5.1 software (Statpoint Technologies, Inc. Warrenton, Virginia, USA). $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The *In vitro* hypoglycemic activity was measured by the methods of glucose adsorption capacity and glucose dialysis retardation index. *C. limetta* peel flour had a glucose adsorption capacity of 16.58 mM showing a similar effect with the positive control (cellulose) which adsorbed 17.43 mM of glucose, without statistical difference when they are compared, indicating that they have the same effect [Figure 1]. This capacity may be attributed to its fiber content such as fiber from different sources able to adsorb glucose.[15]

The glucose dialysis retardation test is useful to predict the effect of the samples on the delay in glucose absorption in the gastrointestinal tract.[16] In this study, glucose diffusion rates of *C. limetta* peel flour were time dependent. The GDRI increased considerably from 1 to 3 h, *C. limetta* peel flour and cellulose (positive control) showed significant inhibitory effects on glucose movement after the second hour, and both samples kept similar values at the three measurement times, without statistical difference among them. The higher GDRI was found at 3 h with 33.79% for *C. limetta* peel flour and 34.55% for cellulose [Table 1]. This result

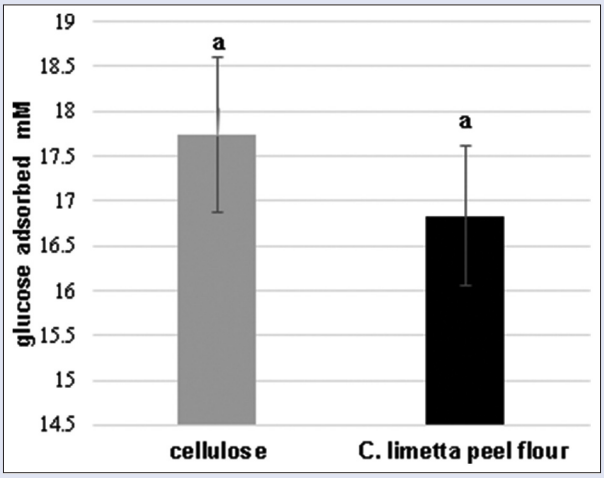

Figure 1: Adsorption of glucose. Cellulose had a capacity of 17.43 ± 0.86 mM of glucose and *Citrus limetta* peel flour a capacity of 16.58 ± 0.77 mM of glucose. Assays were performed in triplicate. Bars with same letters do not differ statistically from each other, $P < 0.05$

Table 1: Glucose dialysis retardation

Treatment	1 h (mm/L)	GRDI (%)	2 h (mm/L)	GRDI (%)	3 h (mm/L)	GRI (%)
Negative control	1.1±0.1	0	1.6±0.2	0	2.2±0.1	0
Citrus limetta peel	0.94±0.13	14.85	1.14±0.03[a]	32.5	1.44±0.06[a]	33.79
Cellulose	0.86±0.08	22.12	1.08±0.19[a]	28.75	1.45±0.03[a]	34.55

[a]Values significantly (P<0.05) different from negative control but without statistical difference among *Citrus limetta* peel flour and cellulose. Assays were performed in triplicate. GDRI: Glucose dialysis retardation index; GRI: Glucose retardation index

revealed that *C. limetta* peel flour might be efficient in retarding the glucose absorption. Similar effects have been observed for other citrus peels.[17-20]

It is known that citrus peel has high-fiber content and some studies have revealed that fiber can help lowering postprandial serum glucose levels. Three mechanisms have been proposed: the viscosity increase in the intestine to hinder the glucose diffusion, glucose uptake, and the retardation of α-amylase activity.[13,14,21] Taking into account our previous study which revealed that *C. limetta* peel flour has high ability to inhibit α-glucosidase and α-amylase and now the *in vitro* results showed capacity to glucose adsorption and its retardation, we suspected that *C. limetta* peel flour sample plays a role in lowering postprandial serum glucose level in the intestinal lumen as other fibers.[18]

Therefore, it was carried out a test to show if *C. limetta* peel flour played a role in lowering postprandial serum glucose levels. The results of this test showed that the animal group treated with *C. limetta* peel flour showed blood glucose levels of 41.4 mg/dL and that value was significantly lower than the negative control group which had a value of 120 mg/dL; meanwhile, mice treated with positive control (acarbose) showed a glucose level of 51.7 mg/dL. There was no statistical difference between *C. limetta* peel flour and acarbose values [Figure 2]. Our results coincide with the reported by Kundusen *et al.*,[22] whereby a methanolic extract obtained from *C. limetta* showed antihyperglycemic effectiveness in streptozotocin-induced diabetic rats.

A great variety of natural products inhibits the enzymes responsible for carbohydrate digestion due to the presence of compounds such as terpenes, flavonoids, alkaloids, phenylpropanoid, among others. Recently, Barreca *et al.*[23] identified eight glycosylated flavonoids compounds in *C. limetta* juice, and it has been suggested that these types of compounds are related to antihyperglycemic and antioxidant activity. It is known that the presence of flavonoids in therapeutic plants, confers antioxidant, antibacterial, anti-inflammatory, among other properties.[2,24,25]

In addition to these beneficial properties, several studies indicate that content of phenolic compounds is higher in the peel in relation to other sections of the citrus fruit. The peel acts as a protective barrier; in the fact that, it is outside, favors the synthesis of phenolic compounds involved in the antihyperglycemic activity, and is also a source of antioxidants.[2,5,25,26]

The safety of plants is of vital important when they are used clinically; therefore, we tested the acute toxicity in mice, finding that all animals showed good tolerance at the dose of 2000 mg/kg. The treated mice did not show noticeable signs of toxic effects on behavior or appearance, and all mice survived during the whole experimental period, and also, the body weight and food consumption were normal during the study period, which indicate that the use of *C. limetta* peel flour would be safe because the study did not show toxic effects.

CONCLUSIONS

C. limetta peel flour is able to decrease the concentration of blood glucose after consumption as we demonstrated in this study. It is considered that the hypoglycemic activity of *C. limetta* peel flour is due to its absorption in the intestine, which was corroborated by *in vitro* and *in vivo* assays. Lime peel is a byproduct that could act as a source of functional compounds in the treatment of DM and enhance the activity of synthetic oral hypoglycemic drugs. Natural products are a good alternative with broad benefits, within these few or null side effects. However, it is necessary to perform assays with flour lime peel using an induced diabetic model and finding bioactive molecules responsible of hypoglycemic effect in *C. limetta* peel flour.

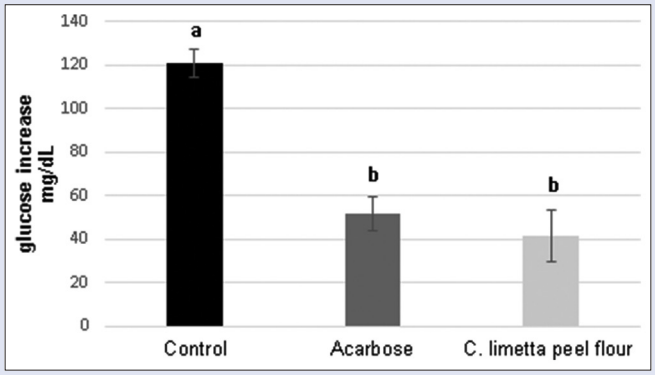

Figure 2: Postprandial hypoglycemic activity. Glucose levels in mice serum 30 min after hyperglycemia induced by maltose. Each of the three groups was treated with the vehicle, acarbose (30 mg/kg), and *Citrus limetta* peel flour (300 mg/kg). Assays were performed in triplicate. Bars with different letters denote statistically significant differences from each other, $P < 0.05$

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Conflicts of interest

There are no conflicts of interest.

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