

## Article

# Hypoglycemic and Antihyperglycemic Potential of Flavonoid Fraction from *Citrus sinensis* (L.) Osbeck in Normoglycemic and Diabetic Rats

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**Abstract:** Diabetes is one of the most prevalent diseases worldwide, and the search for therapeutic alternatives in developing countries has been focused on natural products, primarily from plants. This study evaluated the antihyperglycemic and hypoglycemic activities of the albedo (FA) and flavedo (FF) flavonoid fractions obtained from orange peels (often discarded) in normoglycemic Wistar rats. The flavonoid fractions were identified and quantified using HPLC-UV-DAD and compared with glibenclamide, repaglinide, saxagliptin, and acarbose. Additionally, both fractions were tested in a streptozotocin (65 mg/kg)/nicotinamide (100 mg/kg)-induced diabetic model. In normoglycemic rats, the highest glucose variation (%VG) occurred during the first hour after FA (112.8%) and FF (105.30%) administration at 100 mg/kg, indicating a hypoglycemic effect. In diabetic rats, FF at 100 mg/kg showed the highest %VG (140.41%) during the first hour after administration. HPLC-UV-DAD analysis revealed the presence of hesperidin (HSP) and naringenin (NGN), with the highest concentrations found in FA (HSP: 41.41%; NGN: 10.75%). These findings suggest potential antihyperglycemic effects of FA and FF fractions, possibly attributed to the presence of HSP and NGN. The results obtained in this work lay the foundations to explore the therapeutic applications of orange peels for controlling hyperglycemia in diabetes. In conclusion, our results suggest a reevaluation and revalorization of orange peels, as they contain pharmaceutically relevant flavonoids.

**Keywords:** type 2 diabetes; traditional medicine; hesperidin; naringenin; orange fruit

## 1. Introduction

Mayan regions are renowned for their rich herbal and plant diversity, traditionally used in various medicinal practices. This valuable indigenous knowledge presents a significant opportunity to explore the metabolism of these medicinal plants, leading to the discovery of natural products with potent biological activities and a deeper understanding

of their pharmacological and chemical properties [1]. *Citrus sinensis*, commonly known as sweet orange, holds particular importance among these plants. Belonging to the Rutaceae family, it is widely distributed in tropical and subtropical zones, with a global production exceeding 110 million tons annually, making it an excellent source for obtaining secondary metabolites on a large scale [2,3].

The traditional use of *Citrus sinensis* in treating various health issues, such as digestive disorders, cancer, diuretics, cardiovascular diseases, diabetes, and antibacterial and antiviral conditions, highlights its potential therapeutic activities primarily associated with its flavonoid content [4–6].

Flavonoids have been widely investigated for their medicinal properties and have shown promise as adjuncts in treating complex diseases like type 2 diabetes (T2DM). The World Health Organization (WHO) reports that 80% of the global population relies on medicinal plants as their primary healthcare option, underscoring the significance of exploring plant extracts and secondary metabolites for pharmacological activities [7].

However, despite the therapeutic potential of antidiabetic plants, further in-depth research is essential to enhance their safety and efficacy in vivo. The pharmacological mechanisms of these plants are often not fully understood, and the complex mix of several metabolites in herbal formulations may exert their effects by regulating multiple metabolic pathways and targeting various proteins or receptors [8].

Based on the traditional uses of *Citrus sinensis* and the growing research on flavonoids in chronic degenerative diseases such as diabetes, it was hypothesized that orange peels could be a potential and privileged source of bioactive flavonoids for diabetes treatment. Therefore, the present study evaluates the flavonolic fraction content in the exocarp and mesocarp parts of *Citrus sinensis* to explore their potential hypoglycemic and antihyperglycemic effects in normoglycemic and diabetic rats. Using HPLC-UV-DAD, we aim to identify the primary metabolites in the flavonolic fraction, shedding light on the potential therapeutic benefits of this natural resource. By doing so, we hope to contribute to the growing body of knowledge surrounding the pharmacological potential of medicinal plants and pave the way for developing new and effective treatments for diabetes and related conditions.

## 2. Materials and Methods

### 2.1. Chemical and Reagents

Hesperidin (HSP) and naringin (NGN) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). The solvents (HPLC grade) for HPLC analysis were supplied by Sigma-Aldrich Co. (St. Louis, MO, USA). Glibenclamide (GBC) (Silanes<sup>®</sup>, Jamestown, RI, USA), repaglinide (RPG) (Prandin<sup>®</sup>, Novo Nordisk, Bagsvaerd, Denmark, Sanfer<sup>®</sup>, Mexico City, Mexico), saxagliptin (SAX) (Onglyza<sup>®</sup>, Astra Zeneca, Cambridge, UK/Bristol-Myers Squibb), acarbose (ACB) (Glucobay<sup>®</sup>, Bayer Shering Pharma, Leverkusen, Germany), and metformin (MET) (Merck<sup>®</sup>, Rahway, NJ, USA) were used as a control in pharmacological activity evaluation, and all of them were solubilized in water solution of NaCl, at 0.9% (*w/v*).

### 2.2. Plant Material and Extraction

*Citrus sinensis* (L.) Osbeck National variety was collected in Akil, Yucatan, Mexico (20°14' and 20°22' N; 89°18' and 89°26' W) in December 2009. Flavedo (FF) and albedo (FA) were the plant materials used. Flavonoids were obtained from the dried and ground albedo (210 g) extracted with a Soxhlet apparatus with n-hexane (3 L). The same plant material was extracted with methanol (MeOH, 3 L) for 18 and 20 h, respectively. For flavedo, 425 g of dried and ground material were used under the same extraction conditions, but only for 14 h with n-hexane and 15 h with methanol. The n-hexane fraction was discarded, and the methanolic fraction was evaporated to dryness under reduced pressure. The extract was resuspended in 100 mL of MeOH at 0 °C and sonicated in an ultrasonic bath for 30 min with ice. Finally, the solution was stored at −20 °C for 24 h. We filtered the methanolic

solution at  $-20\text{ }^{\circ}\text{C}$  with a Whatman<sup>®</sup> filter (Cytiva, Marlborough, MA, USA) (No 1). The precipitate was washed with cool methanol at  $4\text{ }^{\circ}\text{C}$  until precipitation. The precipitates obtained were marked as FA and FF.

### 2.3. Hesperidin and Naringenin Quantification in *C. sinensis* Using HPLC-UV-DAD

HSP and NGN quantification in FA and FF was performed in an Agilent 1200 series (Agilent Technologies, San Jose, CA, USA) equipped with a C<sub>18</sub> column of  $250 \times 4.6\text{ mm}$  i.d.  $5\text{ }\mu\text{m}$  (Waters Speherisorb<sup>®</sup>, Milford, MA, USA) and UV-DAD detector. The chromatographic method was developed in gradient mode using acetic acid  $10.5\text{ mmol/L}$  (A) and acetonitrile (B). The first step of the gradient started at 0–5 min, 80 to 60% of A, then to 5–8 min, 60 to 40% of A, and finally to 8–12 min, 40% of A, with a flow of  $1.0\text{ mL/min}$  and injection volume of  $20\text{ }\mu\text{L}$ . The calibration plot was obtained between  $12.5$  and  $50\text{ mg/mL}$ . The concentration ( $\text{mg/mL}$ ) was calculated by linear regression and the percentage (%) of each flavonoid in the extracts. The maximum absorbance was  $280\text{ nm}$  with a retention time of  $5.896$  and  $6.087\text{ min}$  for HSP and NGN, respectively.

### 2.4. Quality Control Parameter

To establish the quality control of the chromatographic method, we calculated the following parameters: capacity factor ( $k'$ ), number of theoretical plates (N), efficiency (H), resolution ( $R_s$ ), and selectivity ( $\alpha$ ) for HSP-NGN. The linearity evaluation of the system required knowing the relationship between the concentration of each solution and its area. In addition, it was necessary to determine the calibration curve's correlation coefficient ( $r$ ). The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the standard deviation attributable to the blank solution ( $S_{bl}$ ) using the following equation:

$$\text{LOD or LOQ} = (k \cdot S_{bl}) / \sqrt{n} \quad (1)$$

### 2.5. Extraction Yield and HPLC-UV-DAD Quantification

The flavonols fraction was extracted with a Soxhlet apparatus, with FA and FF yields  $2.34$  and  $0.33\%$  ( $w/w$ ), respectively. These fractions were evaluated in HPLC-UV-DAD to quantify HSP and NGN; the chromatographic method showed an adequate resolution between each signal, capacity factor ( $k'$ ), and excellent column efficiency tested using several theoretical plates (H), which is an acceptable value to develop a solid quantitative analysis. Furthermore, the limit of detection (LOD) and limit of quantification (LOQ) were observed between  $0.27$  and  $0.81\text{ ng/L}$  for NGN and  $0.12$  and  $0.39\text{ ng/L}$  for HSP (Table 1). These results were made using a calibration curve between  $12.5$  to  $50\text{ mg/L}$ .

**Table 1.** Chromatographic parameter values for each flavonoid standard.

Parameter	NGN	HSP
w <sup>a</sup>	0.100	0.101
$k'$	1.36	1.44
N	$5.5 \times 10^4$	$5.8 \times 10^4$
H <sup>b</sup>	$4.51 \times 10^{-4}$	$4.30 \times 10^{-4}$
$R_s$		1.88
$\alpha$		1.06
LOD (ng/L)	0.27	0.12
LOQ (ng/L)	0.81	0.39

<sup>a</sup> Value of w, taken from the base of the peak and expressed in min; <sup>b</sup> values in cm/plate; LOD: detection limit; LOQ: quantification limit.

Both flavonol fractions were resuspended in HPLC-grade methanol at  $100\text{ mg/mL}$  and tested in HPLC-UV-DAD to quantify NGN and HSP, showing a concentration ( $\text{mg}/100\text{ g}$  of dry material) in FACsN was  $961.45 \pm 4.82$  and  $251.26 \pm 6.66$ , respectively. In FF  $118.60 \pm 0.27$  and  $33.15 \pm 0.55$  of HSP and NGN, respectively, the results indicated an HSP/NGN ratio of 4:1 in both samples. Likewise, the ratio for FA/FF is 8:1. However,

in 2006, Nogata et al. [9] reported an HSP/NGN ratio of 300:120 in the bark of *C. sinensis* grown in Japan. This variation in the values obtained may be due to climatic factors. Similarly, the HSP/NGN ratio of FA/FF was 1:1.

## 2.6. Animals

Male Wistar rats between 250 and 300 g of weight at around ten weeks old were obtained from the Animal House of the Centro de Investigaciones Regionales “Dr. Hideyo Noguchi”, “Universidad Autónoma de Yucatán (CIR-UADY)”. The animals’ diet consisted of a standard rodent diet with *ad libitum* water. The temperature of the animal house was controlled at  $25 \pm 3$  °C. The light cycle was for 12 h of light and 12 h for darkness, with a humidity of around 50–65%. All experiments were conducted according to NOM-062-ZOO-1999 national regulation from Mexico and were approved by an ethical committee from the Research Bioethics Committee of CIR-UADY.

## 2.7. Oral Glucose Tolerance Test (OGTT) in the Normoglycemic Rat Model

We used normoglycemic rats based on Ortiz-Andrade et al. in 2007 [10]. Before each experiment, rats were fasted for 16 h without water deprivation. Each experimental group contained five animals; all the extracts were dissolved in a water solution of 0.9% (*w/v*) of NaCl and were administered via an intragastric route. The first group was the control group; in the second and third, we administered 50 mg/kg of FA and FF separately; in the fourth and fifth, we administered 100 mg/kg of FA and FF separately. The hypoglycemic reference groups were GBC (10 mg/kg), RPG (4 mg/kg), ACB (10 mg/kg), SAX (10 mg/kg), and MET (120 mg/kg). Following Ortiz-Andrade et al. [10], the glucose measurement started after administering glucose solution (2 g/kg) at 0, 0.5, 1, 2, 3, and 4 h. We used a commercial glucometer (Accu-Chek Active, Roche®, Basel, Switzerland) to measure blood glucose and plotted the variation glycemia for each group, considering the initial value at 0 h, according to:

$$\text{Variation of glycemia (VG\%)} = \frac{G_x - G_0}{G_0} \times 100 \quad (2)$$

where  $G_0$  was the initial glycemia value, and  $G_x$  was the glycemia value at +0.5, +1, +2, +3, and +4 h, respectively [11].

## 2.8. OGTT in T2DM-Induced Rat Model

Based on Ortiz-Andrade et al. in 2007 [10], we induced diabetic physiological and metabolic conditions in the rats using Streptozotocin (STZ) and nicotinamide (NDA) by intraperitoneal injection of 65 mg/kg STZ 15 min after *i.p.* administration of NDA 100 mg/kg. After fifteen days, elevated plasma glucose levels confirmed T2DM, considering a fasting plasma glucose concentration above 150 mg/dL. The above protocol was used to evaluate the antidiabetic effect of FA and FF. The rats were fasted for 16 h before the experiments, with free access to water. The FA and FF were dissolved in water at 0.9% (*v/w*) of NaCl and dosed intragastrically at 100 mg/kg (groups 2 and 3).

Due to its pharmacologic mechanism, we used GBC (10 mg/kg) as a hypoglycemic reference drug (group 4). The control group only received water solution at 0.9% (*v/w*) of NaCl as a vehicle in a dosage of 0.5 mL/100 g. To evaluate the extracts, we waited 15 min after administering the extract to administer a dose of 2 g/kg of glucose intragastrically to each rat. We followed the OGTT normoglycemic rat model steps and measured the glucose simultaneously with the same equipment. Each group’s percentage variation in glycemia was calculated according to Equation (2). A hypoglycemic effect was considered when the percent glucose values (%VG) were significantly lower than the baseline value. Conversely, when %VG was significantly lower than those of the control group but higher than the baseline level, an antihyperglycemic effect was considered; however, both can occur.

### 2.9. OGTT in T2DM-Induced Rat Model

Based on Ortiz-Andrade et al. [10], we induced diabetic physiological and metabolic conditions in the rats using Streptozotocin (STZ) and nicotinamide (NDA) via intraperitoneal injection of 65 mg/kg STZ 15 min after i.p. administration of NDA 100 mg/kg. After fifteen days, elevated plasma glucose levels confirmed T2DM, considering a fasting plasma glucose concentration above 150 mg/dL. The above protocol was used to evaluate the antidiabetic effect of FA and FF. The rats were fasted for 16 h before the experiments with free access to water. The FA and FF were dissolved in water at 0.9% (*v/w*) of NaCl and dosed intragastrically at 100 mg/kg (groups 2 and 3).

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### 2.10. Statistical Analysis

All experimental values were expressed as mean  $\pm$  standard error. We used Microcal<sup>TM</sup> Origin 8.0 (Microcal Software Inc., Los Angeles, CA, USA) to calculate the values using ANOVA one-way analysis. After that, if statistical differences ( $p < 0.05$ ) were found, Tukey test was used as a post hoc analysis. Figures were generated using the GraphPad Prism version 8.0 (GraphPad Software, Inc., San Diego, CA, USA).

## 3. Results

### 3.1. Extraction Yield and HPLC-UV-DAD Quantification

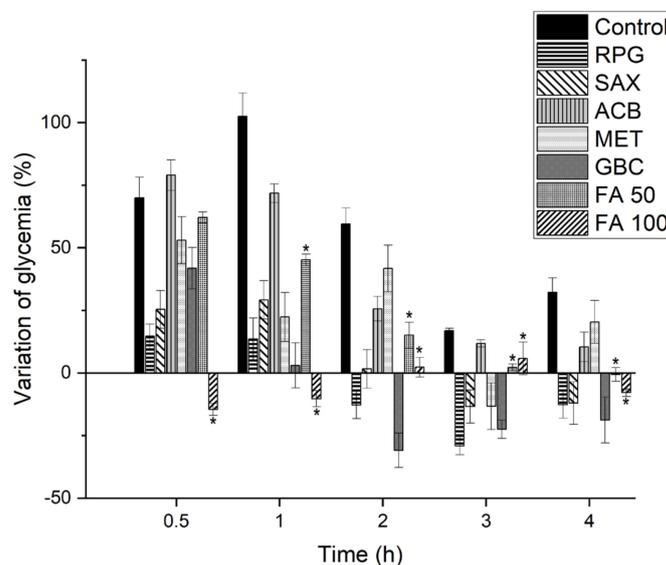
The flavonoid fraction was extracted using a Soxhlet apparatus, resulting in FA and FF yields of 2.34% and 0.33% (*w/w*), respectively. Both fractions were subjected to HPLC-UV-DAD analysis, demonstrating adequate resolution between each signal, capacity factor ( $k'$ ), and excellent column efficiency tested by several theoretical plates ( $H$ ). These results indicate an acceptable value for robust quantitative analysis. The limit of detection (LOD) and limit of quantification (LOQ) for NGN and HSP were found to be between 0.27 and 0.81  $\mu\text{g/L}$  and 0.12 and 0.39  $\mu\text{g/L}$ , respectively (Table 1). These values were determined using a calibration curve between 12.5 to 50  $\mu\text{g/L}$ .

For further quantification, both flavonoid fractions were resuspended in HPLC-grade methanol at 100  $\mu\text{g/mL}$  concentration and analyzed using HPLC-UV-DAD. The concentration of NGN and HSP in FA was found to be  $961.45 \pm 4.82$  mg/100 g of dry material and  $251.26 \pm 6.66$  mg/100 g of dry material, respectively. In FF, the concentrations of HSP and NGN were  $118.60 \pm 0.27$  mg/100 g of dry material and  $33.15 \pm 0.55$  mg/100 g of dry material, respectively. The results indicated an HSP/NGN ratio of 4:1 in both samples, and the ratio of FA/FF was 8:1.

### 3.2. Antihyperglycemic/Hypoglycemic Effect of *C. sinensis* Fractions on Rat Models

The effect of FA administration on the oral glucose tolerance test (OGTT) was evaluated, and the results are presented in Figure 1. At a 100 mg/kg dose, FA demonstrated a significant hypoglycemic effect, which commenced within the first half-hour post-administration (%VG:  $-14.6$ ) and persisted until the fourth hour (%VG:  $-7.9\%$ ). In contrast, a dose of 50 mg/kg induced an antihyperglycemic effect, starting in the first hour post-administration (%VG ISS: 102.5% vs. %VG FA: 45.2%), which was sustained until the second hour (%VG ISS: 59.5% vs. %VG FA: 15.0%). Notably, the most substantial differ-

ence in blood glucose variations between the experimental and control groups occurred in the first hour (%VG ISS–%VG FA: 112.8%) with the 100 mg/kg dose. Subsequently, the effect seemed to transition back to the remaining hypoglycemic effect. However, the dose-dependent effects did not follow the same trend observed in the OGTT at 100 mg/kg.

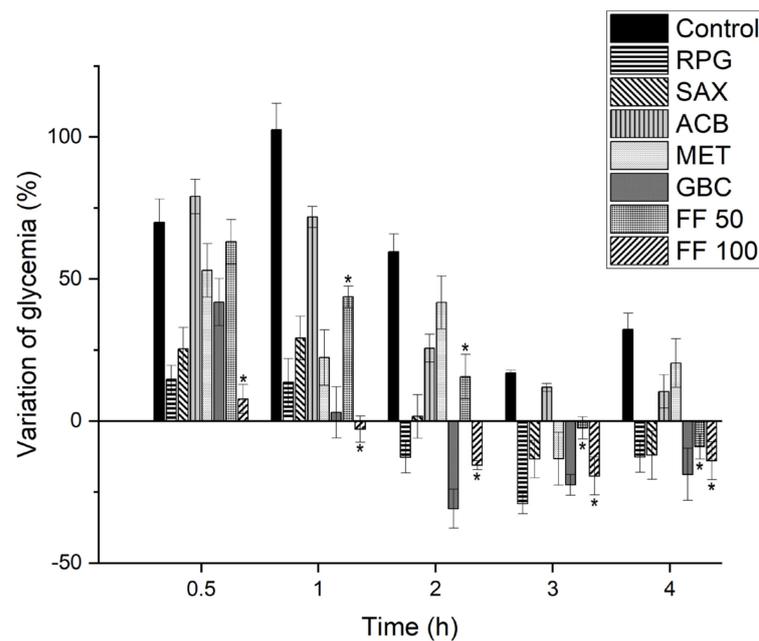


**Figure 1.** Percentage change in glycemia after oral administration of FA (50 mg and 100 mg per kg; dark gray) in normoglycemic rats compared to reference antidiabetic drugs (light gray). Values are expressed as the mean of %VG  $\pm$  standard error,  $n = 5$  animals. Asterisk (\*) means statistical significance ( $p \leq 0.05$ ) compared to the control group. Repaglinide (RPG) 4 mg/kg; Saxagliptin (SAX) 5 mg/kg; acarbose (ACB) 5 mg/kg; metformin (MET) 120 mg/kg; glibenclamide (GBC) 5 mg/kg.

The effects of FA were also compared with those of known antidiabetic drugs with established mechanisms of action. At a 100 mg/kg dose, FA exhibited a stronger effect during the first half-hour after administration than the secretagogue drugs (GBC and RPG). However, the effect during the second and third hours was less pronounced than that of the reference drugs, while no significant difference was observed in the fourth hour post-administration. Conversely, there were no notable differences between FA (100 mg/kg) and the antihyperglycemic drugs ACB ( $\alpha$ -glucosidase inhibitor), SAX (DPP-IV inhibitor), and MET (insulin sensitizer) during the fourth hour post-administration. Interestingly, when FA (50 mg/kg) was compared to these antihyperglycemic drugs, its behavior resembled that of SAX (with lower potency) and ACB (with higher potency). This suggests that, at this dose, the extracts may exert an antihyperglycemic effect through delayed glucose absorption.

The effect of FF administration on OGTT assessment was also investigated. In Figure 2, we observed a hypoglycemic effect induced by the administration of 100 mg/kg (although it did not reach negative values of %VG, it can be considered an antihyperglycemic effect). This lower hypoglycemic effect commenced during the first half-hour after administration (%VG: 7.7%) and intensified until the fourth hour post-administration (%VG:  $-14.0\%$ ).

Furthermore, a potential antihyperglycemic effect was evident at a dose of 50 mg/kg, persisting until after the first hour post-administration (%VG ISS: 102.5% vs. %VG FF: 58.8%). This effect extended to the second hour post-administration (%VG ISS: 59.5% vs. %VG FF: 15.6%), displaying a slight hypoglycemic behavior from the third hour post-administration (%VG:  $-2.5\%$ ), which continued until the end of the experiment (%VG:  $-8.9\%$ ). Importantly, this effect showed a dose-dependent relationship, and unlike FA, both doses exhibited a consistent trend in their OGTT performance.



**Figure 2.** Percentage change in glycemia after oral administration of FF (50 mg and 100 mg per kg; dark gray) in normoglycemic rats compared to reference antidiabetic drugs (light gray). Values are expressed as the mean of %VG ± standard error, n = 5 animals. Asterisk (\*) means statistical significance ( $p \leq 0.05$ ) compared to the control group. Repaglinide (RPG) 4 mg/kg; Saxagliptin (SAX) 5 mg/kg; acarbose (ACB) 5 mg/kg; metformin (MET) 120 mg/kg; glibenclamide (GBC) 5 mg/kg.

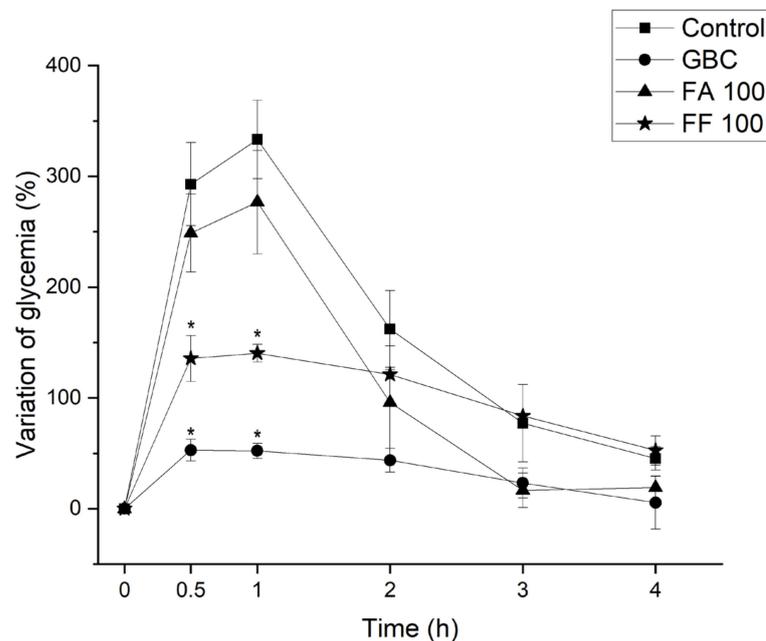
Moreover, the effects of FF administration were compared with those of oral antidiabetic drugs during the OGTT assessment. At a 100 mg/kg dose, the effect during the first half-hour post-administration was more pronounced than that produced by GBC or RPG. However, this effect was not observed at a dose of 50 mg/kg. For both doses, the effect during the second and third hours post-administration was lower compared to the control drugs. At the same time, there was no significant difference during the fourth hour post-administration.

In the case of the two drugs used as controls, their effect is attributed to stimulating insulin secretion, which is initiated within the first half-hour after administration. Notably, both drugs displayed a similar response trend, except for RPG, which exhibited a more potent effect in the first hour post-administration. Interestingly, we observed a behavior comparable to RPG at a 100 mg/kg dose of FF, with slightly more pronounced effects during the first two hours post-administration. This observation may suggest a potential mechanism of insulin secretion, as this mechanism facilitates a faster reduction in plasma glucose levels.

Furthermore, when comparing the effects of FF with antihyperglycemic drugs, both doses displayed a similar trend to SAX and ACB, with the only exception being the case of the 50 mg/kg dose. The effect was more potent than ACB at this dose but less so than SAX. Although this mechanism cannot be definitively verified based solely on OGTT data, it may provide valuable insights into this extract’s potential mechanism of action at this specific dose (Figure 2). Further investigations are needed to fully elucidate the underlying mechanisms and confirm the efficacy of FF as an antihyperglycemic agent.

After evaluating the hypoglycemic/antihyperglycemic effect in normoglycemic rats, both extracts were further assessed in STZ/NDA-induced diabetic rats. In this model, a 100 mg/kg dose was administered for both extracts, and we used GBC (5 mg/kg) as the positive control. Figure 3 illustrates the antihyperglycemic effect of FF, which began during the first half-hour post-administration (%VG ISS: 292.9% vs. %VG FF: 135.6%) and persisted until the first hour (%VG ISS: 333.4% vs. %VG FF: 140.4%). However, the effect

disappeared in the second hour (%VG ISS: 162.2% vs. %VG FF: 121.4%), but it was observed again until the end of the experiment (%VG ISS: 45.4% vs. %VG FF: 52.6%).



**Figure 3.** Oral glucose tolerance test (OGTT) for FA 100 mg/kg (FA 100) and FF 100 mg/kg (FA 100) in NA/STZ-induced diabetic rats. Values are expressed as the mean of %VG  $\pm$  standard error,  $n = 5$  animals. Asterisk (\*) means statistical significance ( $p \leq 0.05$ ) compared to the control group. Glibenclamide (GBC) 5 mg/kg.

As for FA, no significant differences in %VG values were observed between the experimental group and the control ISS, starting from the first half-hour post-administration (%VG ISS: 292.9% vs. %VG FA: 248.9%) and continuing until the end of the experiment (%VG ISS: 45.4% vs. %VG FA: 18.9%). This suggests that a higher dose of FA may be necessary to observe a significant effect in diabetic rats. Moreover, it is worth noting that the oral glucose administration in the diabetic rats might have masked the hypoglycemic effect, as even in control GBC, we did not observe negative %VG values throughout the evaluation. Further investigations are warranted to explore the optimal dosages and potential mechanisms underlying the antihyperglycemic effects of both extracts in the diabetic rat model.

FA and FF exhibited significant hypoglycemic and antihyperglycemic effects in both evaluated models. At doses of 50 mg/kg, the fractions displayed an antihyperglycemic profile, while at 100 mg/kg, the plasma glucose concentration curve indicated a hypoglycemic-type effect in normoglycemic rats. Furthermore, it is essential to highlight that only FF demonstrated antihyperglycemic activity, which suggests a potential correlation with its higher concentration of HSP. These findings emphasize the therapeutic potential of both fractions as effective regulators of blood glucose levels and underscore the importance of HSP content in contributing to the antihyperglycemic properties observed in FF. Further studies are warranted to elucidate these flavonoid-rich fractions' underlying mechanisms and potential applications in managing hyperglycemia in various pathological conditions.

#### 4. Discussion

Diabetes has become one of the most concerning diseases worldwide, doubling the number of people affected in the last decade. According to the World Health Organization (WHO) report, there are now 422 million people living with diabetes globally (WHO, 2022). Polyphenols are crucial in carbohydrate and glucose metabolism, controlling various metabolic processes. Specific effects of polyphenols have been documented in inhibiting

enzymes related to the hydrolysis of complex carbohydrates, such as  $\alpha$ -glucosidases and  $\alpha$ -amylase, leading to a reduction in the intestinal absorption of glucose [12]. They also increase pancreatic insulin secretion, enhance the ability of muscles and adipocytes to utilize glucose, and prevent the liver from secreting glucose [13].

Our findings reveal that orange peels are a significant source of polyphenols, particularly hesperidin and naringenin, with the albedo tissue being the richest in these compounds. Both fractions demonstrated significant hypoglycemic and anti-hyperglycemic effects in both evaluated models. Therefore, this effect can be attributed to hesperidin and naringenin, as they were the predominant compounds observed by HPLC-UV-DAD.

Regarding hesperidin, it is primarily found in citrus waste. In the context of pharmacological effects related to diabetes, it has been studied as a neuromodulator for reducing oxidative stress. Its high antioxidant effect has been notably highlighted in mitigating damage caused by streptozotocin (STZ) in induced murine diabetes models [14]. Its impact on diabetic neuropathy has also been investigated. A study by Wang et al. (2011) demonstrated that hesperidin exerts a neuroprotective effect on peripheral nerves in streptozotocin-induced diabetic rats [15]. Furthermore, it has been compared to aminoguanidine, which inhibits diamine oxidase and nitric oxide synthase, thus preventing diabetic neuropathy by inhibiting the non-enzymatic glycation of tissue proteins. In this regard, Hesperidin has exhibited effects in reducing the onset of diabetic neuropathy.

Diabetes, a condition that disrupts metabolic equilibrium, often affects various organs, including the heart and kidneys. In this context, research on hesperidin has revealed its antiapoptotic impact by upregulating the Bcl-2 protein expression and downregulating the biosynthesis of the Bax protein in ischemia-reperfusion injuries within diabetic rats [16]. Furthermore, this flavonoid has shown the potential to bind with and activate PPAR- $\gamma$ , explaining hesperidin's diverse effects as a flavonoid with antidiabetic, anti-inflammatory, and antioxidant properties [17]. Highlighting the pharmacological and medicinal properties of hesperidin, its capacity to modulate inflammation-related cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, is noteworthy. Moreover, its significant antioxidant power is closely linked to mitigating oxidative stress. Given that diabetes is a multifactorial disease often connected to inflammatory and cellular oxidation processes, hesperidin emerges as an excellent candidate for therapeutic intervention in this context [18].

Akiyama et al. (2010) reported that in a murine model of streptozotocin-induced diabetes, hesperidin successfully reduced both blood glucose and insulin levels. Furthermore, it normalized the activity of critical enzymes such as glucose-6-phosphatase, glucokinase, and various hepatic enzymes that tend to elevate diabetes [19]. Our results align with these findings, as we observed that hesperidin, the predominant compound in the extract, could exert pharmacological effects like those described above. As seen in our results, at time intervals of 0.5 to 1.5 h, a significant decrease in glycemic variation was noted at a dose of 100 mg/kg. This suggests a potential control over glucose metabolic pathways, promoting glucose metabolism within muscles.

Observing a reduction in plasma glucose levels within 0.5 h at a 100 mg/kg dose also implies a mechanism that modulates insulin secretion. This is notable, given that other metabolic pathways usually do not exhibit such a phenomenon quickly. Insulin secretion and the subsequent increase in its plasma concentration have been reported by Visnagri et al. (2014), who determined that hesperidin yielded positive effects on various biochemical parameters related to diabetes, such as cholesterol and triglycerides. Additionally, it contributed to the decrease in glucose levels mediated by insulin secretion [20].

In the STZ-induced diabetes model, damage to pancreatic  $\beta$ -cells leads to hypoinsulinemia and hyperglycemia, two severe factors disrupting glucose metabolism homeostasis. In our results (Figure 3), we observe how administering glibenclamide, a well-known insulin secretagogue, achieves a maximum glycemic variation of around 50%. In comparison, the FFCsN extract reaches 150%, in contrast to the control group with 350%. Considering the study model and the damage caused by STZ, it is reasonable that the FFCsN extract has a pharmacological activity through a mechanism similar to glibenclamide, inducing

or promoting the availability of serum insulin; previously, we highlighted the insulin secretion mediated by hesperidin [20]. This has also been linked to the attenuation of hyperglycemia-mediated oxidative stress, where hesperidin can suppress the production of cytokines like TNF- $\alpha$  and IL-6 in type 2 diabetic rats induced by HFD/STZ [21].

The mechanism of action of hesperidin in the STZ-induced diabetes model was elucidated by Mahmoud et al. (2015), demonstrating its ability to reduce fasting glucose levels and attenuate insulin resistance in diabetic rats. It also enhances insulin release in isolated pancreatic islets [22]. Moreover, it normalizes the activities of metabolic enzymes, such as glucose-6-phosphatase, glycogen phosphorylase, and fructose-1,6-bisphosphatase. Furthermore, it has been proposed that hesperidin increases the expression of mRNA and proteins of GLUT4 in adipose tissue, facilitating improved glucose metabolism and consequently reducing serum glucose levels [23].

Like many polyphenolic compounds, Naringenin, another flavonoid isolated from oranges, exhibits numerous activities. However, its research focus has predominantly centered on its anti-inflammatory and antioxidant properties [24]. Notably, naringenin has not been extensively studied in the context of diabetes but rather in diseases linked to diabetes or that can contribute to its development, such as obesity and metabolic syndrome. In these contexts, naringenin has shown the ability to reduce the expression of Mac-2 gene mRNA, a crucial macrophage marker, though it did not yield significant effects on glucose levels [25]. This flavonoid has exhibited effects in obese models with insulin resistance, highlighting its impact on inflammation through cytokine modulation and the suppression of neutrophil infiltration in adipose tissue [26]. While its specific effects on diabetes are not as extensively explored, naringenin's potential in addressing related conditions, its influence on inflammation, and its association with key markers make it an intriguing candidate for further investigation in diabetes research.

## 5. Conclusions

The flavonoid-rich fractions, FA and FF, isolated from *C. sinensis*, demonstrated significant antihyperglycemic and hypoglycemic activities in normoglycemic rats at 50 and 100 mg/kg doses, with FF exhibiting notable antihyperglycemic effects in diabetic rats as well at a 100 mg/kg dose. The observed hypoglycemic effects are likely attributed to their high HSP content and potential modulation of insulin secretion. The findings underscore the valuable potential of *C. sinensis* peels as a traditional alternative for managing type 2 diabetes. Overall, our study contributes valuable insights into the pharmacological potential of *C. sinensis* peel as a source of flavonoids with antihyperglycemic and hypoglycemic activities.

**Author Contributions:** Conceptualization: J.A.A.-L. and R.O.-A.; methodology: D.C.-S., P.V.-G., Á.C.-W., A.A.G.-S., I.F.-N. and Z.M.-Q.; formal Analysis: J.A.A.-L., R.O.-A. and J.C.S.-S.; investigation: D.C.-S., P.V.-G., Á.C.-W., A.A.G.-S., I.F.-N. and Z.M.-Q.; data curation: M.R.S.-C., J.U.-N. and Z.M.-Q.; resources project administration: R.O.-A., visualization: J.A.A.-L., R.O.-A., M.R.S.-C. and J.U.-N.; writing—original draft: J.A.A.-L., R.O.-A. and J.C.S.-S.; writing—review and editing: D.C.-S., P.V.-G., Á.C.-W., A.A.G.-S., I.F.-N., M.R.S.-C., J.U.-N., J.C.S.-S. and Z.M.-Q. All authors have read and agreed to the published version of the manuscript.

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