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*Original Research Article*

# Antioxidant, Antiglycation, Inhibition of Digestive Enzymes and Enhanced Glucose Uptake Activities of *Opuntia ficus indica* L, *In Vitro* and *In Vivo*

Rhizlan Abdnim[1]*, Hayat Ouassou[2], Amal Elrherabi[1], Nour Elhouda Daoudi[1], Ali Berraaouan[1], Abdelkhaleq Legssyer[1], Abderrahim Ziyyat[1], Hassane Mekhfi[1], Mohamed Bnouham[1]*

[1]*Laboratoire de bioressources, biotechnologie, ethnopharmacologie et santé, Département de biologie, Faculté des sciences, Université Mohamed premier, Boulevard Mohamed VI ; BP : 717 ; 60000, Oujda, Maroc.*
[2]*Higherinstitute of nursing professions and health techniques, Oujda 60000, Morocco*

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ABSTRACT

*Opuntia ficus indica* L (Cactaceae), is commonly employed in Morocco as an alternative medicinal approach for managing diabetes mellitus (DM). The objective of the study is to explore and examine novel mechanism underlying the antidiabetic activity of *Opuntia ficus indica* seed oil (OFIO), which has shown promising potential in managing diabetes, but the exact mechanisms responsible for its antidiabetic effects remain unclear. Therefore, the current study explores the antihyperglycemic effectof OFIO, the *in vitro* antioxidant properties, and the antiglycation activity of OFIOof hemoglobin (Hb) model. The total phenolic levels were determined using Folin–Ciocalteu colorimetric approach and total flavonoid levels were determined using colorimetric assay with aluminum chloride. The effect of antihyperglycemia was evaluated using the inhibitory effect of pancreatic α-amylase activity both *in vitro* and *in vivo* using Alloxan induced-diabetic rats and the glucose uptake in an isolated rat hemidiaphragm *in vitro* model. The antioxidant activity was studied using the DPPH Radical scavenging activity test. In addition, the antiglycation activity of OFIO was also tested *in-vitro* using hemoglobin model. The outcomes derived from this research have demonstrated that OFIO exhibited potent α-amylase inhibitory activity *in vitro* ($p < 0.01$; IC50 values of 4.24 ±0.18 mg/ml, and 4.05± 0.08 mg/ml respectively) and in vivo using Alloxan induced-diabetic rats. In contrast, OFIO has no effect on glucose uptake by the rat diaphragm muscle as compared to negative control. In addition, OFIO showed a significant anti-oxidant and hemoglobin antiglycation activities ($p<0.001$) with an IC50 value of 2.05± 0.06 mg/ml, and 0.485± 0.017 mg/ml, respectively.The findings of this study suggest that is OFIO has promising antidiabetic activity.

***Keywords***: *Opuntia ficus indica*, diabetes mellitus, α-amylase, antiglycation, antioxidant, hemidiaphragm

## Introduction

Diabetes Mellitus (DM) is a multifaceted metabolic disorder, involving inappropriately elevated blood glucose levelsthat result from a deficiency in insulin production, or action, or both.[1,2] It has emerged as a leading cause of mortality and morbidity on a global scale.[3] In 2019, it was predicted that about 463 million peoples (9.3%) of the whole population suffer from diabetes and the indicated number is projected to reach 578 million (10.2%) in 2030, and 700 million (10.9%) in 2045.[4] The postprandial hyperglycemia is a contributing factor in the evolution of diabetes complications such as retinopathy, nephropathy,diabetic foot ulcerand neuropathy.[5,6]

Uncontrolled high blood glucose levels may produce glycation of proteins that is known as Maillard reaction. It's a non-enzymatic reaction produced by the link between free amino groups of proteins and the carbonyl group from reducing sugars to form an unstable Schiff base. The product then stabilizes over a period of time to give rise to advanced glycation end product (AGEs).[7] Furthermore, the study aims to explore the interaction between advanced glycation end products (AGEs) and their receptors, knows as the receptor for AGEs (RAGE), which leads to an increase in the generation of reactive oxygen species (ROS) and inflammation. These processes contribute to the progression of various vascular diseases linked with diabetes.

A positive correlation between oxidative stress and glycated albumin levels has been described in patients with diabetes mellitus.[8] Many oral antidiabetic medications commonly exhibit secondary effects when used for diabetes treatment. Hence, there are several approaches identified for preventing and managing Diabetes and its associated vascular complications, one of which involves the utilization of medicinal plants.[9] The recent antidiabetic herbal medicine provides a chance to discover natural compounds able of exerting positive impact and avoiding the side effects of synthetic therapeutic agents.

*Opuntia ficus indica* is classified in the Cactaceae family and is grown primarily in the United States, as well as in Mediterranean nations and in desert and semi-arid regions of Mexico. It is often known as "nopal" in Mexico, "prickly-pear cactus" in the Southern United States and "Indian fig cactus" in Europe. *Opuntia ficus indica* have traditionally been used in Eastern popular medicines to treat several illnesses, including diabetes,burns,hypertension, edemaasthma and indigestion.[10,11] This plant is recognized for its abundant levels of vitamins and antioxidants.[12] *Opuntia ficus indica* is utilized for its antioxidant properties and has also been employed in the treatment of diabetes.[13] In Mexico, *Opuntia ficus indica* historically employed for addressing diabetes. [14] A variety of pharmacological activities of the

*Corresponding author. E mail: smbnouham@yahoo.fr
Tel: + (212) 6 67 62 74 96

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stem have been described, such asantioxidant, anti-inflammatory, hypoglycemic and apoptotic activities. [11,15,16,17] It is used to the antidiabetic properties of traditional medicine in Morocco.[18] Consequently, the primary focus of the study is to evaluate the antihyperglycemic and antioxidant potential of *Opuntia ficus indica* seed oil from Eastern Morocco. We conducted assessments to examine the impact of this plant on the glycative activity of hemoglobin *in vitro*. Additionally, we evaluated its α-amylase activity inhibitory activities and how it influences the process of glucose absorption using an isolated rat hemidiaphragm model, both *in vitro* and *in vivo*.

## Materials and Methods

*Chemical and reagents*

The used drugs and solvents are starch (Sigma-Aldrich, Germany), acarbose and gallic acid (Sigma-Aldrich, china), α-Amylase enzyme (Sigma-Aldrich, USA), D (+) glucose (Riedel-de Haën, Germany), diethyl ether (Somaprol, Casa-Blanca, Morocco), Alloxan monohydrate and 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich, China), Folin-Ciocalteau (Sigma-Aldrich, Switzerland)., Tween-20(Sigma Aldrich, St.Louis, MO, USA),Sodium Carbonat and Aluminum chloride (Sigma-Aldrich, Germany). Methanol and Petroleum (Riedel-de Haën, Poland), Gallic acid and Rutin (Sigma Aldrich, Dorset, UK), acarbose (Sigma Aldrich, St. Louis, MO, USA), glucose oxidase–peroxidase (GOD–POD) (Biosystems, Barcelona, Spain), Dinitrosalicylic acid (Sigma Aldrich, Riedel-de Haen, Germany), the chemicals were of analytical quality.

*Plant material*

The fruits of *Opuntia ficus indica* (Cactaceae) were sampled from Guercif region (North-Eastern Morocco) in September 2021. The identification of the vegetative material was done in the Scientific Institute of Mohammed 5 University of Rabat (Rabat, Morocco) by the expert botanist Mohammed Fennan. A voucher specimen HUMPOM 826 has been placed in the Herbarium of the Faculty of Sciences, University Mohamed First, Oujda, Morocco.

Initially, the biomass was obtained by mechanically ground using an electric crusher, the biomass sample weighing 100 g was used. The extraction of the biomass was carried out through a method called maceration. In this process, the biomass was mixed with petroleum ether in a ratio of 1:5 (100 g of biomass in 500 ml of petroleum ether) for 24 h. After obtaining the fusion solution, it underwent filtration and evaporation using a rotary evaporator set at 40°C. The resulting oil was stored in a refrigerated at 4°C until use.

*Animals*

Wistar rats weighing 150–250 g, both sexes of animals (♂/♀). Were used for this experiment. The animals were provided by the animal house of the department of biology, Faculty of Sciences, Oujda, Morocco. Animals were housed under standard laboratory conditions in a well ventilated animal house and maintained at room temperature (24 ± 2°C) under 12 h light/12 h gloomy cycle with complimentary availability of food and water. Animals were handled in compliance with the internationally accepted guide for the care and use of laboratory animals, published by the US national institutes of Health, is being upheld (Guideline, 2011).

*The total phenolic level determination*

To estimate the total phenolic levels in *Opuntia ficus indica* oil (OFIO), the Folin Ciocalteau reagent was employed, adopting the methodology outlined by by Hagerman, 1988 in with a few adjustments. [19] In brief, 500 µl of OFIO (1g/mL, dissolved in methanol solvent) was added to 250 µL of follin-ciocalteu solution (0.2 N), prosecuted by adding of 500 µL of sodium carbonate ($Na_2CO_3$, 2%). The mixture has been maintained to stay longer than 90 min in the gloom at ambient temperature, and the measurements of absorbance were taken at 750 nm. A standard solution of gallic acid was prepared with varying concentrations to create a concentration gradient 0.25, 50, 75, 125, 250 and 500 µg/Ml. The total phenolic level was quantified using a calibration curve, and the findings were conveyed as milligrams of gallic acid equivalent (GEA) per 100 grams of dry weight. Every sample was made in triplicate.

*The total flavonoid level determination*

The total flavonoid level of OFIO was determined by slight modification of the colorimetric method described by Ayoola et al., 2008. [20] A total of 1mL of $AlCl_3$ solution (dissolved in 2% of methanol) was added to 1 mL of OFIO in test tubes. The composite solution was incubated over a period of 30 minutes at ambient temperature in the dark. We read the absorbance at 415 nm compared with methanol blank. The results were adjusted once the rutin standard was carried out in the same manner (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/mL), and were reported as mg rutin equivalent per 100 g dry weight. Three replicates of each sample were prepared for each analysis, and the average absorbance value was determined.

*DPPH-Radical scavenging activity of OFIO*

OFIO's free radical scavenging activity has been tested with the aid of a 1,1-diphenyl2-picryl hydrazyl (DPPH) technique, as declared by Shahidi, 2011, with some modifications. [21] Summarily, 0.2 ml of OFIO at adiverse concentration (0.15, 0.31, 0.62, 1.25, 2.5 and 5 mg/mL) were combined with 1,8 mL of DPPH free radical solution (0.001 %). After that, the blend has been incubated and conserved in complete darkness for 30 minutes. The absorbance was therefore assessed at 517 nm with a UV-VIS spectrophotometer. The standard reference has been Ascorbic acid. All samples were performed three times (n =3). This formula was used to establish the Radical scavenging activity percent (RSA):

% of antioxidant activity = [(Ac- As)/Ac] × 100

Where: Ac: Control reaction absorbance; As: Sample reaction absorbance

*Determination of antiglycation activity, in vitro*

The anti-glycation activity was conducted following the methodology outlined by Nair et al. in 2013, with slight modifications.[22] In summary, 25 µL of *Opuntia ficus indica* oil (OFIO) at a concentration of 80 mg/mL was blended with 1 mL of a 5% hemoglobin solution. Additionally, 5 µL of gentamicin at a concentration of 40 mg/mL was added to each tube to prevent bacterial infection. A glucose solution at a concentration of 4 mg/mL (1 mL) subsequently, it was introduced or included in the mixture. The reaction mixture was kept in darkness for 72 hours. Positive controls were prepared using varying concentrations of gallic acid (2.5, 5, 10, 20, 40, and 80 mg/mL). The absorbance was measured at 443 nm.

The anti-glycation activity of the samples was derived by applying the following formula for calculation:

% of inhibition = [1 - (Abs blank - ((Abs Control - Abs Sample) / Abs Control))] × 100

In this experiment, the following measurements were taken:

Abs blank: Absorbance of hemoglobin without any sample or glucose.

Abs control: Absorbance of hemoglobin mixed with glucose.

Abs sample: Absorbance of hemoglobin mixed with glucose and the sample being tested.

*In vitro, inhibited α-amylase activity*

The inhibitory activity of α-amylase was conducted using a modified version of the method originally described by Thalapaneni et al. in 2008. [23] A total of 100 µL of phosphate buffer (0.2 M, pH 6.9), 100 µL of α-amylase enzymatic solution (13 IU), and 100 µL of either OFIO (at concentrations of 0.9, 2.25, 4.5, and 9 mg/mL) or acarbose (at concentrations of 0.45, 0.9, 2.25, and 4.5 mg/mL) were mixed together. This mixture was then preincubated at 37°C for 10 minutes. After the preincubation, 100 µL of a 1% starch solution (dissolved in phosphate buffer) was added to the reagent mixture. The resulting mixture was subjected to incubation at a temperature of 37°C for a duration of 20 minutes. Upon completion of the incubation period, the reaction was stopped by adding 300 µL of a colored reagent called 3,5 dinitrosalicylic acid (DNSA). To develop color intensity, the reaction mixture-filled tubes were immersed and incubated in a hot water bath at 100°C for 8

minutes. Following this, they were cooled in a frozen water bath for 5 minutes. The reagent mixture was diluted with 1 mL of distilled water, and the absorbance of the solution was measured at a wavelength of 540 nm. By performing these steps, the inhibitory activity of α-amylase was determined based on the absorbance measurement at 540 nm. To quantify the inhibitory activity of α-amylase, the following equation was employed:

$$\% \text{ Inhibition} = [(\text{Abs Control} - \text{Abs Samples})]/(\text{Abs Control}) \times 100$$

*In vivo, inhibited α-amylase activity*

*Diabetes induction*

Wistar rats were subjected to an overnight fast (16 hours) and subsequently induced into a diabetic state by a single intraperitoneal injection of alloxan (120 mg/ml) dissolved in a phosphate sodium-citrate buffer (0.2 M, Ph 3). After one week of the alloxan injection, plasma glucose levels were assessed by obtaining blood samples from the tail. Heparinized capillary tubes were used to collect the blood, and a small incision was made at the tail tip to facilitate the blood collection. The blood glucose level was measured using the glucose oxidase peroxidase (GOD-POD) method. Rats exhibiting fasting blood sugar levels above 1.5 g/l were selected for further experimentation.

*Experimental design*

Wistar rats with diabetes, weighing between 150 and 250 grams, were divided into four groups through a random allocation process, each consisting of six animals (n=6, ♂/♀=1).

Group 1 served as the control group and received oral administration of distilled water at a dose of 10 mL/kg.

Group 2 received oral treatment with acarbose, a reference drug, at a concentration of 10 mg/kg per kilogram of body weight.

Group 3 was treated with OFIO at a dose of 1ml/kg body weight (921 mg/kg).

Group 4 was administered OFIO at a dose of ml/kg body weight (1842 mg/kg).

Thirty minutes after the respective treatments, all animals were orally given a starch solution at a dosage of 2 g/kg. Blood samples were collected from the tail vein of the rats at 0, 30, 60, and 120 minutes following the starch administration to measure blood glucose levels. The measurement was carried out using the glucose oxidase peroxidase (GOD-POD) method.

*Peripheral glucose consumption of OFIO*

To assess peripheral glucose uptake in rat hemidiaphragm, the procedure described by Bnouham et al. in 2010 was followed with some minor modifications. Rats weighing between 150 and 250 grams were divided into five groups, with each group consisting of six animals (n = 6; ♂/♀ = 1). The groups were treated as follows:

Group1: The Tyrode glucose solution (1 g/L) was incubated.

Group2: The Tyrode glucose solution (1 g/L) was incubated along with insulin (4 UI/mL).

Group3: The Tyrode glucose solution (1 g/L) was incubated along with tween 40 (1%).

Group4: The Tyrode glucose solution (1 g/L) was incubated along with insulin (4 UI/mL) and tween 40 (1%). Group5: The Tyrode glucose solution (1 g/L) was incubated along with OFIO (1 g/L).

After fasting the normal rats for 36 hours, they were lightly anesthetized, followed by cervical dislocation. An incision was made in the abdominal wall, and the diaphragms were dissected and divided into two equal halves. These hemidiaphragms were then placed in sample tubes containing the tyrode solution and incubated for 1 hour at 37°C with constant oxygenation (95% O2 and 5% CO2), while shaking at 120 cycles per minute.

The isolated rat hemidiaphragms were separated, dried for 2 hours at 40°C, and weighed to obtain their dry weight. The glucose uptake per gram of dry hemidiaphragm was determined by measuring the difference between the starting and ending blood glucose levels in the incubated environment. The glucose consumed was measured using the glucose oxidase peroxidase (GOD-POD) method.

*Statistical analyses*

The data were represented as means ± standard errors. Statistical analysis was conducted using GraphPad version 5 for windows software, developed by San Diego, CA, USA. To determine significant differences among multiple groups compared to the control group, a one-way analysis of variance (ANOVA) with Dunnett's post hoc test was performed. A probability level of $p< 0.05$ was considered statistically significant.

## Results and Discussion

*Determination of Total Phenolic and Flavonoid levels of OFIO*

The concentration of phenolic and flavonoid compounds in OFIO was determined. The standard curves for gallic acid and rutin were established with regression equations of $y = 0.0084x$ ($R^2 = 0.9971$) and $y = 0.1785x$ ($R^2 = 0.9916$), respectively. Based on these equations, the total phenolic content of OFIO was found to be 0.115 ± 0.28 mg of gallic acid equivalents (GAE) per 100 grams of dry weight (DW). Additionally, the total flavonoid content was measured to be 8.658 ± 3.71 mg of GAE per grams of DW (Table 1). These results indicate that OFIO has a higher concentration of flavonoids compared to polyphenols.

*DPPH-Radical scavenging activity of OFIO*

The results of the radical-scavenging activity of OFIO was expressed as percent inhibition of DPPH radical "Fig 1". Indeed, the DPPH test of *Opuntia ficus indica* seed oil showed a significant raise ($p< 0.001$) of the antioxidant activity from (61.23±7.002 %) to (83.63 ± 1.67%) with the increase of extract doses from 0.15 to 5 mg/mL. In the same way, ascorbic acid showed an important DPPH radical scavenging activity ($p< 0.001$), which was (94,90 ± 1,41%) to (95,17 ± 1,98 %) at the 0.15 µg/mL to 5 µg/mL respectively. This antioxidant activity was statistically significant. in comparison with the control. Furthermore, the $IC_{50}$ DPPH radicals of OFIO was 2.05 ± 0.06 mg/mL. Comparability, the standard ascorbic acid presented $IC_{50}$ value of 1.77 ± 0.03 mg/mL against DPPH radicals "Table 2". These values showed the dose-dependent effect of OFIO on the anti-radical power.

The results of the radical-scavenging activity of OFIO (*Opuntia ficus indica* seed oil) were presented as the percentage inhibition of the DPPH radical (Figure 1). The DPPH test demonstrated a significant increase ($p< 0.001$) in antioxidant activity for OFIO, ranging from 61.23 ± 7.002% to 83.63 ± 1.67%, as the extract doses increased from 0.15 to 5 mg/mL. Similarly, ascorbic acid exhibited notable DPPH radical scavenging activity ($p< 0.001$), with percentages ranging from 94.90 ± 1.41% to 95.17 ± 1.98% at concentrations of 0.15 µg/mL to 5 µg/mL, respectively. These antioxidant activities were statistically significant compared to the control group.

Furthermore, the IC50 value of DPPH radicals for OFIO was determined as 2.05 ± 0.06 mg/mL, whereas ascorbic acid exhibited an IC50 value of 1.77 ± 0.03 mg/mL (Table 2). These values indicate a dose-dependent effect of OFIO on its anti-radical power.

**Table 1:** Total phenolic and flavonoid contents of *Opuntia ficus indica* seed oil (OFIO) (n = 3)

| Extract | Species | Total Polyphenols (mg GAE/100 gDW) | Total Flavonoids (mg Rutin/100g DW) |
|---|---|---|---|
| Seed Oil (OFIO) | *Opuntia ficus indica* | 0.115 ± 0.28 | 8.658 ± 3.71 |

Values are expressed as mean ± SEM

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**Table 2:** $IC_{50}$ values (mg/mL) of DPPH scavenging capacity by OFIO and ascorbic acid.

| | *Opuntia ficus indica* seed oil (OFIO) (n = 6) | Ascorbic acid (n = 6) |
|---|---|---|
| DPPH radicals | 2.05 ± 0.06 | 1.77 ± 0.03 |

Values are expressed as mean ± SEM

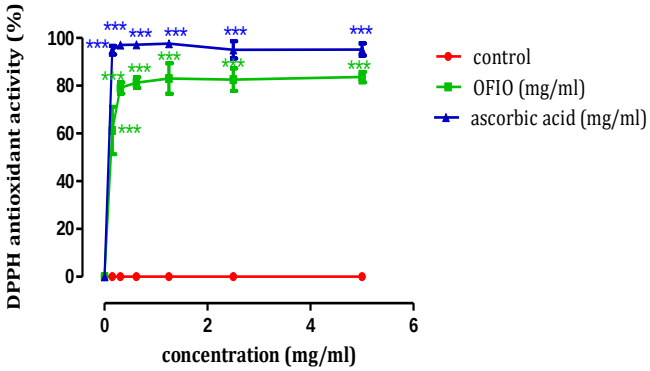


**Figure 1:** DPPH-Radical scavenging activity of *Opuntia ficus indica* seed oil (OFIO). Each value is reported in mean ± SEM (n = 3). *** $p< 0.001$ as a function of the control group.

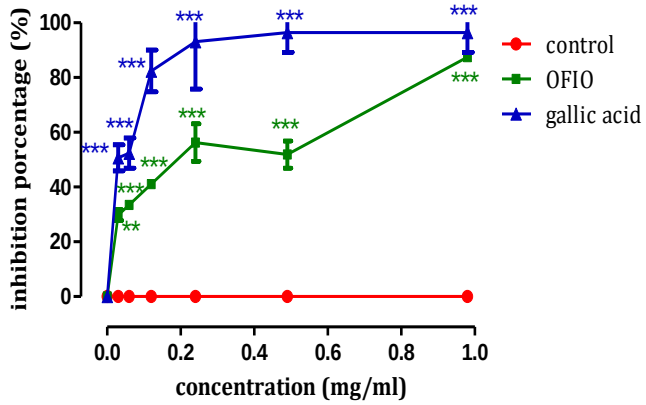


**Figure 2:** inhibitory effect of OFIO and Gallic acid (positive control) on glycation activity of hemoglobin. Results are shown as mean ± SEM (n=3). ** $p< 0.01$; *** $p < 0.001$ as function of the control group.

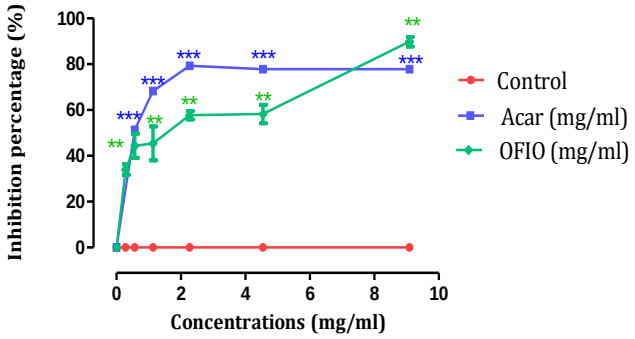


**Figure 3:** Inhibitory effectof α-amylase enzymes through OFIO (*opuntia ficus indica* seed oil) and Acar (acarbose) *in vitro.* The values are the means ± SEM (n = 3). ** $p< 0.01$, *** $p < 0.001$ as function of the control group.

*In vitro anti-glycation activity of OFIO*

OFIO (*Opuntia ficus indica* seed oil) was tested for its antiglycation activity at various concentrations ranging from 0.03 to 0.98 mg/mL. The results revealed that OFIO significantly inhibited the glycation activity of hemoglobin compared to the control group (without the addition of oil), with a high level of statistical significance ($p< 0.001$). Notably, OFIO exhibited the highest percentage activity (87.46 ± 0.83%) at a concentration of 0.98 mg/mL (Figure 2).

The IC50 value of OFIO indicated its significant antiglycation activity, with a value of 0.485 ± 0.017 mg/mL. Furthermore, gallic acid displayed the highest percentage of antiglycation activity (96.45 ± 5.46%) at a concentration of 0.98 mg/mL ($p< 0.001$), and its IC50 value was determined to be 0.362 ± 0.015 mg/mL (Table 3). These findings highlight the potential antiglycation activity of OFIO, with gallic acid serving as a reference standard with even higher activity.

*In vitro, α-amylase activity inhibition*

In Figure 3, the impact of OFIO (*Opuntia ficus indica* seed oil) on the activity of α-amylase *in vitro* is depicted. Different doses of OFIO (0.9, 2.25, 4.5, 9 mg/mL) were tested for their inhibitory effects on α-amylase. The results revealed a dose-dependent inhibition of the enzyme activity by OFIO, indicating a competitive type of inhibition. The highest percentage of inhibition (87.46 ± 0.83%) was observed at a concentration of 9 mg/mL. In comparison, the standard drug displayed an inhibition of 79.34 ± 1.64% at a dose of 2.25 mg/mL.

Additionally, the IC50 values were calculated for OFIO and the reference drug acarbose, resulting in values of 4.24 ± 0.18 mg/mL and 4.05 ± 0.08 mg/mL, respectively (Table 4). These findings suggest that OFIO exhibits α-amylase inhibitory activity, and its potency is comparable to that of acarbose, a commonly used α-amylase inhibitor.

*In vivo, inhibition of pancreatic α-amylase activity*

In the oral starch tolerance tests, it was observed that groups receiving a dosage of 1 mL/kg of OFIO exhibited a significant decrease ($p< 0.001$) in postprandial hyperglycemia at 30 minutes (0.85 ± 0.01 g/L) and 60 minutes (0.86 ± 0.008 g/L) compared to the control group given distilled water (10 mL/kg). Similarly, groups treated with a dosage of 2 mL/kg of OFIO showed a significant reduction ($p< 0.001$) in postprandial hyperglycemia at 30 minutes (0.76 ± 0.03 g/L) and 60 minutes (0.69 ± 0.05 g/L) compared to the control group. Additionally, acarbose at a dosage of 10 mg/kg significantly decreased ($p< 0.001$) postprandial hyperglycemia at 30 minutes (0.98 ± 0.02 g/L) and 60 minutes (0.95 ± 0.01 g/L) after starch loading compared to the control group (Figure 4A).

The area under the curve (AUC D-glucose) was significantly lower ($p< 0.001$) in rats treated with OFIO (108.03 ± 1.68 g/L/min and 91.85 ± 7.92 g/L/min) compared to rats treated with distilled water (147.63 ± 1.97 g/L/min). Furthermore, the AUC of acarbose was significantly reduced (114.73 ± 2.59 g/L/min) compared to the AUC of the water-treated rats (147.63 ± 1.97 g/L/min) (Figure 4B).

In Alloxan-diabetic rats, treatment with both doses of OFIO (1 mL/kg and 2 mL/kg) significantly reduced the increase in glucose levels after the starch load at 30 minutes ($p< 0.05$; 3.19 ± 0.74 g/L and 3.53 ± 0.22 g/L) compared to the diabetic control group treated with distilled water, where the starch overload led to elevated blood glucose levels at 30 minutes (4.44 ± 0.26 g/L), 60 minutes (3.97 ± 0.26 g/L), and 120 minutes (4.24 ± 0.24 g/L). Similarly, in the Acarbose-treated group, the glucose level was significantly ($p< 0.01$) reduced at 30 minutes (2.54 ± 0.18 g/L) and 60 minutes (2.55 ± 0.19 g/L) compared to the diabetic control group (Figure 5A).

Moreover, the AUC D-glucose was significantly smaller ($p< 0.05$) in diabetic control rats treated with OFIO (385.31 ± 26.28 g/L/min) compared to diabetic rats treated with distilled water (502.10 ± 42.03 g/L/min). Additionally, the AUC of acarbose was significantly lower ($p< 0.05$) at 341.78 ± 13.48 g/L/min compared to that of diabetic control rats (Figure 5B).

*Peripheral glucose consumption of OFIO, in vitro*

The effect of OFIO on glucose uptake by isolated rat hemidiaphragm is depicted in Figure 6. The result showed that the insulin-treated group (4 IU/mL) significantly improved glucose uptake by isolated rat hemi-

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diaphragm significantly ($p$<0.05; 95,121 ± 24,390 mg/g/h) in compared to the control group (43,902 ± 7,317 mg/g/h). However, the presence of OFIO at a concentration of 1g/L did not show a significant effect (8.536 ± 7.317 mg/g/h) compared to the control group (43.902 ± 7.317 mg/g/h). Likewise, the administration of OFIO and insulin together did not stimulate glucose absorption (12.195 ± 10.975 mg/g/h) when compared to the control group. Tween 20 (1%) utilized as a negative control.

**Table 3:** $IC_{50}$ value (mg/mL) of antiglycation of hemoglobin by OFIO and gallic acid

| | $IC_{50}$ | |
|---|---|---|
| | ***Opuntia ficus indica* seed oil (OFIO) (mg/mL) (n = 3)** | **Gallic acid (mg/mL) (n = 3)** |
| Glycation inhibition reaction assay | 0.485 ± 0.017 | 0.362 ± 0.015 |

Values are expressed as mean ± SEM

**Table 4:** $IC_{50}$ value (mg/mL) of inhibition of α-amylase by OFIO and acarbose

| | $IC_{50}$ | |
|---|---|---|
| | ***Opuntia ficus indica* seed oil (OFIO) (mg/mL) (n = 3)** | **Acarbose (mg/mL) (n = 3)** |
| α-amylase inhibition | 4.24 ± 0.18 | 4.05 ± 0.08 |

Values are expressed as mean ± SEM

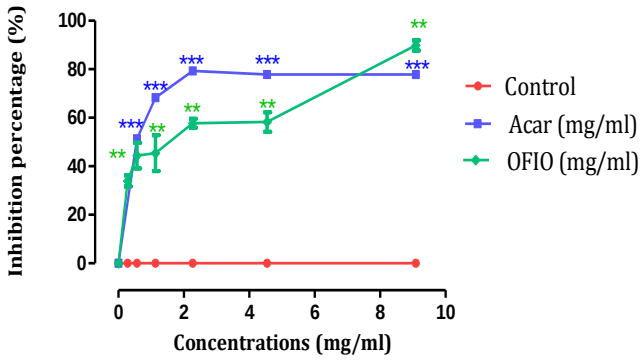


Figure 3: Inhibitory effectof α-amylase enzymes through OFIO (*opuntia ficus indica* seed oil) and Acar (acarbose) *in vitro.* The values are the means ± SEM (n = 3). **$p$< 0.01, *** $p$ < 0.001 as function of the control group

Medicinal plants are used traditionally throughout the globe to treat various diseases including diabetes mellitus, which is gaining high popularity in many countries.[24] *Opuntia ficus indica* seed oil is among the most antidiabetic herbal remedies used in Morocco.[17] This work aimed to evaluate several aspects of the seed oil from OFIO, including phenolic and flavonoid contents, antioxidant activity, hemoglobin antiglycation, inhibition of pancreatic α-amylase, and glucose consumption in isolated rat hemidiaphragm. The outcomes of this investigation revealed that OFIO had a lower content of polyphenols and a higher content of flavonoids compared to other substances examined. These results could be attributed to the climatic conditions of the growing season and harvest period.[25] In addition, OFIO had the highest capacity to scavenge DPPH and an important antiglycation activity against hemoglobin glycation, which can be related to the presence of phenolic substances and flavonoid. In fact, in long term cases of hyperglycemia in diabetes mellitus, glucose undergoes a non-enzymatic reaction known as glycation, where it forms covalent additions with plasma proteins, resulting in structural modifications to the proteins.[26] The antioxidant activity, as well as the levels of polyphenols and flavonoids, are closely linked to the antiglycation activity. Constant hyperglycemia leads to oxidative stress, which in turn promotes the generation of reactive oxygen species (ROS).[27] ROS invasion directly leads to the formation of protein derivatives, including carbonyls and modified amino acids. The evaluation of antioxidants and their protective effects against free radical-induced damage is widely employed procedure.28 This study demonstrated that OFIO exhibited noteworthy antioxidant activity, which is consistent with previous research. The observed antioxidant activity of OFIO, as assessed through DPPH and β carotene bleaching tests, is likely ascribed to the presence of bioactive compounds, notably polyphenols and flavonoids, present in the sample.[29] Moreover, both polyphenols and flavonoids exhibit a potent inhibitory effect on hemoglobin glycation.

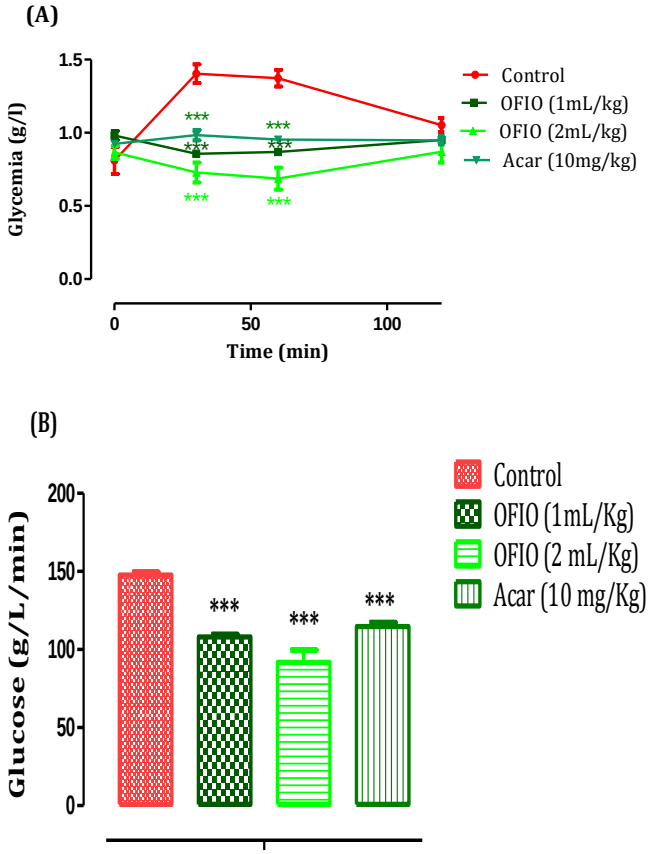


**Figure 4:** Effect of OFIO (1mL/Kg, 2mL/Kg) and Acarbose (10 mg/Kg) on plasma glucose levels after starch intake in normal rats (A), with a representation of the area under the curves (B). The values are the means ± SEM (n = 6). *$p$< 0.05; **$p$<0.01; ***$p$<0.001 compared to the control group. OFIO: *Opuntia ficus indica* oil; Acar: acarbose.

The glycation process, referred to as the Maillard reaction, initiates with the attachment involves the condensation of free amino groups present in proteins with the carbonyl group found in reducing sugars, forming reversible Schiff base compounds. This is followed by a series of chemical reactions that ultimately lead to the formation of advanced glycation end products (AGEs). AGEs are highly reactive and irreversibly form complex structures, which further interact with nearby amino groups of proteins to generate intra- and intermolecular crosslinks.[30,31,32,33] Phenols and flavonoids, known antioxidants, have been suggested to possess inhibitory properties against the generation of advanced glycation end products (AGEs). The presence of hydroxyl (OH) groups in these compounds is believed to contribute to their antioxidant and antiglycation activities.[34,35] In contrast, OFIO demonstrated significant inhibition ofα-amylase activity both *in vitro* and *in vivo* in a dose of 2ml/kg. pre-treatment with OFIO exhibited similar effects to acarbose, a well-known and potent inhibitor of

pancreatic α-amylase. However, the administration of OFIO did not have any effect on glucose consumption by isolated rat hemidiaphragm. *Opuntia ficus indica* is rich of flavonoids content, which are widely considered as alpha amylase inhibitors.[36] Moreover, *Opuntia ficus indica* contains antioxidant compounds such as phenols, flavonoids, betaxanthin, and betacyanin which have been promoted to reflect its health benefits such as hypoglycemia.[37] Furthermore, α-tocopherols and ɣ-tocopherols were high in this plant.[38] Recent studies demonstrated that ɣ-tocopherols prevented alloxan-induced hyperglycemia in mice. [39] In addition, ɣ-tocopherols showed an important antioxidant and anti-inflammatory effects.[40]

## Conclusion

The results revealed that the oil obtained from *Opuntia ficus indica* seed had antioxidant antihyperglycemic activities through inhibiting the digestive enzymes of carbohydrates and hemoglobin glycation. These multiple pharmacological activities, possibly apply to the synergestic effect of its naturally occurring bioactive compounds. This may be useful for the development of new antidiabetic agents from indigenous plant resources.

## Conflict of Interest

The authors declare no conflict of interest.

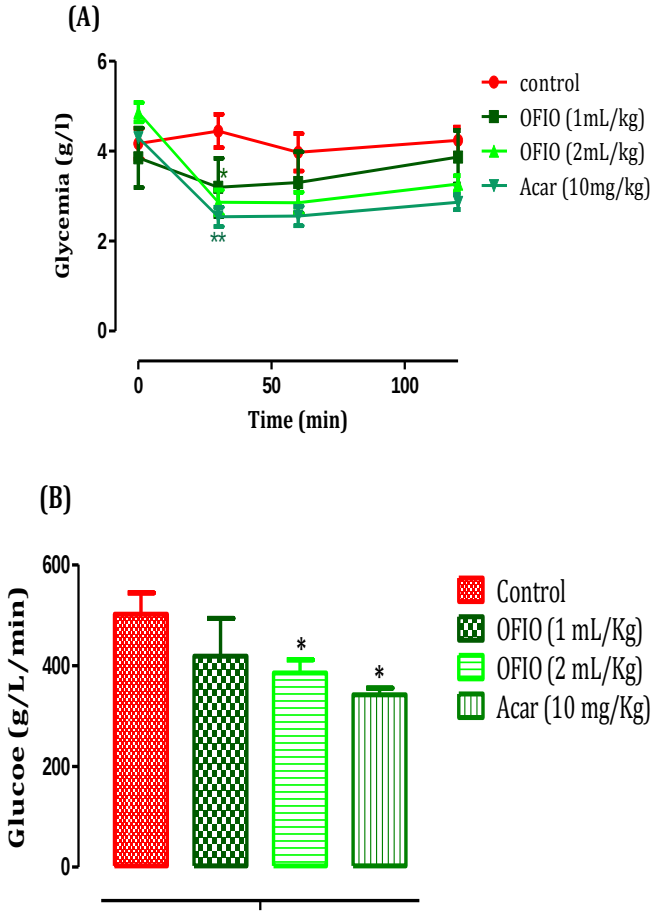


**Figure 5:** Effect of OFIO (1mL/Kg, 2mL/Kg) and Acarbose (10 mg/Kg) on plasma glucose levels after starch intake in diabetic rats (A), with a representation of the area under the curves (B).The values are the means ± SEM (n = 6). *$p$< 0.05; **$p$<0.01 compared to the control group. OFIO: *Opuntia ficus indica* oil; Acar: acarbose.

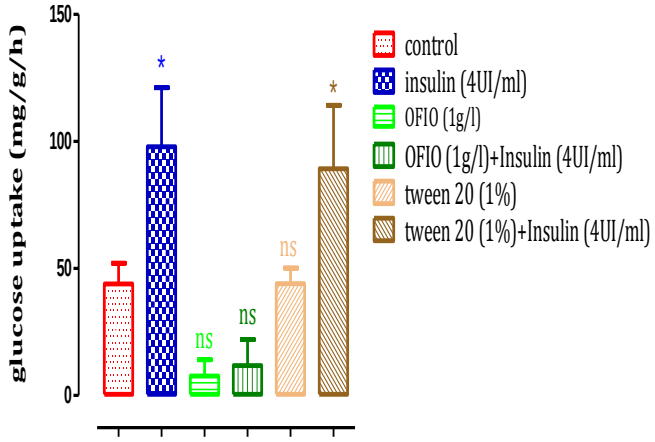


**Figure 6:** OFIO effect on glucose uptake by isolated rat hemidiaphragm. Values are means ± SEM (n = 6). * $p$< 0.05 as function of to the control group. ns: tot significant; OFIO: *Opuntia ficus indica* oil.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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