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Original article

Hepatoprotective effect of *Opuntia microdasys* (Lehm.) Pfeiff flowers against diabetes type II induced in rats

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ABSTRACT

Opuntia sp. has long been used as a folk medicine to treat hepatitis and diabetes in Sicile (Italy). To extract the polyphenols from the flower of *Opuntia microdasys* Lehm. at post flowring stage and evaluate the antidiabetic activity *in vitro* and *in vivo*. The hepatoprotective activity of *Opuntia microdasys* aqueous flowers extract at post flowering stage (OFP) has been tested for their antidiabetic activity. On fructose-alloxan induced diabete in rat model, evaluating the inhibitory effects of OFP on some carbohydrate metabolizing enzymes, pancreatic α-amylase and intestinal α-glucosidase activities *in vitro*. The OFP extract showed inhibitory activity against α-glucosidase ($IC_{50} = 0.17 \pm 0.012$ mg/ml) and α-amylase ($IC_{50} = 2.55 \pm 0.41$ mg/ml). The inhibitory potential of OFP extract on these enzymes suggests a positive and probable role of this extract in the management and treatment of diabetes mellitus, particularly, for type 2. Oral administration of the OFP at 200 mg/kg to diabetic male rats for 28 days demonstrated a significant protective effect by lowering the levels of glucose (123.21 ± 1.38 mg/dL) and hepatic marker enzymes (AST, ALT, LDH, γ-GT, BT, PAL, TC, LDL-C, HDL-C and TG). OFP attenuated oxidative stress by decreasing the SOD, CAT, GP_X activity and the levels of PC and MDA in the liver and restored the histological architecture of the rat liver. OFP has protective effects on the protection of liver, thereby reducing some of the causes of diabetes in experimental animals.

1. Introduction

Diabetes mellitus is one of the major global public health problems. The total number of diabetic peoples was estimated to increase from 171 million in 2000 to 366 million in 2030 and most of these will be dominated by those suffering from type 2 diabetes (95% T2D) [1,2]. T2D is a heterogeneous disorder, characterized by a progressive decline in insulin action (insulin resistance-IR), followed by the inability of pancreatic β-cells to compensate for IR (β-cell dysfunction) [3]. Fructose feeding leads to insulin resistance and compensatory hyperinsulinemia responses [4]. Fasting hyperglycemia is caused by unrestrained basal hepatic glucose output, primarily as a consequence of hepatic resistance to insulin action [5]. Synthetic hypoglycemic agents produce serious side effects, whereas bioactive compounds derived from natural resources are frequently considered safe and cost effective [6]. Thus, plants may play an important role in drug development programs. There is a growing interest among researchers to discover new and effective α-glucosidase inhibitors with minimal side effects, from medicinal plants with known and scientifically proven antidiabetic properties [7,8]. For this reason, the use of natural treatments from food or medicinal plants is considered to be effective and safe for hepatotoxicity, mainly because of the presence of various antioxidant compounds (flavonoid and phenolic acid compounds, etc.). The Cactus (*Opuntia* sp.) has often been used in traditional folk medicine (Cactus pear cladodes, fruits and flowers) for treatment of a great number of diseases and pathological conditions, such as diabetes, stomach ulcers, renal diseases, etc. [9,10]. For example, the traditional Sicilian medicine

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(Italy) uses Cactus flowers as a diuretic agent [11]. The flowers of *Opuntia* have especially been employed as folk remedies for various medical purposes, including the treatment of diabetes [12]. Cactus flowers pass through different stages of ripening (vegetative, initial flowering, full flowering and post-flowering stages) and their chemical composition remarkably vary between these different stages [10]. Our previous studies were focused on the chemical composition of flowers methanol extracts of *Opuntia microdasys* at post floral stage, and their antioxydant activities. This stage demonstrated the ability to scavenge free radicals due to the presence of different antioxidants, mainly phenolic compounds, including *p*-coumaric acid, isorhamnetin-3-*O*-robinobioside, ferulic and caffeic acids derivatives etc. [13]. However, the investigation of effect of the *O. microdasys* flowers, at post floral stage on the glycemia values was not evidenced. The present study aims to investigate the protective effect of the decoction of *Opuntia microdasys*, at post floral stage, *in vitro* and *in vivo* against induced type 2 diabetes (T2D) hepatotoxicity in male *Wistar* rats.

2. Materials and methods

2.1. Plant material

Opuntia microdasys flowers were collected from the cliff of Monastir (Tunisia) at post floral stage [13]. The *O. microdasys* flowers were reduced to a fine dried powder (20 mesh) and mixed to obtain a homogenous sample.

2.2. Preparation of the decoction

For the decoctions preparation, the powder of flowers (100 g) was added to 2 L of boiling distilled water, boiled for 5 min and then left to stand at room temperature for further 5 minutes and filtered through a Whatman filter paper. The obtained decoctions were frozen and lyophilized [14].

2.3. Biochemical assays for determining the inhibition of enzymes activities

2.3.1. α-Amylase inhibition assay

The α-amylase inhibition assay was performed according to the method described by Deguchi et al. [15] with slight modifications. Briefly, the assay mixture consisted of 500 μl of 1% starch solution, 400 μl of 0.1 M sodium phosphate buffer (pH 7.0), 50 μl of plant extract dissolved in dimethyl sulfoxide (DMSO) and 50 μl of pancreatic α-amylase (Sigma, St. Louis, USA) solution (2 U/ml). Then, the reaction medium was incubated at 37 ◦C for 10 min followed by addition of 3 ml of 3,5-dinitrosalicylic acid (DNS) color reagent. Finally, the solution was placed in a boiling water bath for 5 min, diluted with 20 ml of distilled water and the absorbance was measured at 540 nm. Absorbance of a control sample was measured without the plant extract and used as a negative control. The OFP extract was tested for α-amylase inhibitory activity at different concentrations (0.15 to 10 mg/ml). The standard antihyperglycemic agent acarbose was used as a positive control. The results were expressed as percentage inhibition, using the following formula:

$$\text{Percentage inhibition} = ((A_{control} - A_{sample})/A_{control}) \times 100$$

Where $A_{control}$ and A_{sample} are the absorbance values of the negative control and sample, respectively. IC_{50} value, defined as the sample concentration (mg/ml) at which 50% inhibition of the enzyme activity occurs, was calculated from the graph plotting enzyme inhibition against sample concentration. All tests were carried out for three sample replications and the results were averaged.

2.3.2. α-Glucosidase inhibition assay

Enzyme activity was measured following the procedure previously described by Tao et al. [16], with some modifications as detailed by Rengasamy et al. [17]. The α −glucosidase reaction mixture, contained 2.5 mM 4-*p*-nitrophenyl-α-D-glucopyranoside (4-*p*NPG), 250 μL of OFP decoction at different concentrations in DMSO and 0.3 U/mL of α-glucosidase in phosphate buffer pH 6.9, was incubated in a water bath at 37 °C for 15 min. Control tubes contained only DMSO, enzyme and substrate, while in positive controls Acarbose replaced the plant extracts. Absorbance of the resulting *p*-nitrophenol (pNP) was determined at 405 nm and was considered directly proportional to the activity of the enzyme. The OFP extract was tested for α-glucosidase inhibitory activity at different concentrations (0.15 to 5 mg/ml). Percentage inhibition by extracts and acarbose (I %) were calculated using the following formula:

$$\text{Percentage inhibition} = ((A_{control} - A_{sample})/A_{control}) \times 100$$

Where $A_{control}$ and A_{sample} are the absorbance values of the negative control and sample, respectively. The 50% inhibition concentration (IC_{50}, mg/ml) of flowers decoction extract against intestinal α-glucosidase was calculated. All tests were carried out for three sample replications and the results were averaged.

2.4. Animal acclimatization

Sixteen male adult *Wistar* rats (180–200 g) aged 12–14 weeks, were purchased from Central Pharmacy of Tunis, Tunisia. Animals were housed for 2 weeks before experiments and maintained under standard environmental conditions (Temperature: 22 ± 2 °C, relative humidity: 55 ± 5% and 12 h dark/light cycle). Water and a standard aliment were available *ad libitum*. The animals were handled according to the guidelines of the Tunisian Society for the Care and Use of Laboratory Animals, and the study was approved by the University of Tunisia Ethical Committee (approval number: FST/LNFP/Pro 152012). After the acclimatization period 42 rats were employed for experimental study of diabetes.

2.5. Experimental Design

A total of 42 rats, segregated into 7 groups of 6 animals each, were used in this experimentation (C^-: control group, F3C1: rats receiving only the decoction of flower at 100 mg/kg/day, F3C2: rats receiving only the decoction of flower at 200 mg/kg/day, C+: diabetic rats, Met: diabetic rats treated by metformin at 100 mg/kg/day, F3C1: diabetic rats treated by the decoction of flower at 100 mg/kg/day and F3C2: diabetic rats treated by the decoction of flower at 200 mg/kg/day). The four groups C^+, Met, D + F3C2 and D + F3C1 were supplied with 30% fructose solution for 2 weeks to launch the experimentation of the diabetes mellitus type 2, insulin-resistance to glucose [18]. Then animals were fasted for 24 h and made diabetic by a single injection of a freshly prepared solution of alloxan (60 mg/kg body weight) given via tail vein. Animals were allowed to drink 0.5% of glucose solution overnight to overcome the hypoglycemy [19]. The blood glucose level was checked before and 72 h after alloxan injection to confirm the development of diabetes. Only those which showed blood glucose levels >200 mg/dL were separated and used for the study. The diabetic animals were stabilized for five days and the experiment was started on the next day (day 0). Group C^+ served as diabetic control: they daily received only 2 ml of physiological saline; Group Met: diabetic rats: received metformin 100 mg/kg body weight;