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Aqueous extract of *Ocimum tenuiflorum* decreases levels of blood glucose in induced hyperglycemic tilapia (*Oreochromis niloticus*)

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ABSTRACT

Objective: To evaluate, in hyperglycemic tilapia [*Oreochromis niloticus (O. niloticus)*], the effect of this aqueous extract on blood glucose levels. **Methods:** The hyperglycemia in *O. niloticus* was induced by adding glucose to fish pond water. An aqueous extract of *Ocimum tenuiflorum* (*O. tenuiflorum*) was prepared by boiling fresh leaves and the doses of 0, 40, 80, 200 and 400 mg per liter of pond water were tested. **Results:** The blood sugar concentration for tilapia with hyperglycemic induced was an average of 50% higher than the control group. The blood glucose levels in tilapia after the induction of hyperglycemia were higher than the control group for 90 min after the treatment. The treatment with the aqueous extract of *O. tenuiflorum* dropped the serum glucose level of hyperglycemic tilapia until it was similar to that of the control group and was dose dependent. **Conclusions:** The results indicated that *O. tenuiflorum* was endowed with anti-hyperglycemic activity. To our knowledge, this is the first report on the use of fish as a diabetes model to test natural extracts from plants.

1. Introduction

Currently there are over 346 million diabetics worldwide and this number is likely to increase in a 50% or more by the year 2020 due to increases in a sedentary lifestyle, consumption of energy rich diets, and obesity[1]. Providing modern medical healthcare across the world is still a distant goal due to economic constraints. Thus, it is necessary that we continue to look for new, and if possible, more efficacious drugs and phytotherapy may be an ideal target[2].

Although natural supplements are widely used around the world to treat diabetes, few of them have been scientifically validated[3]. Validating the effectiveness of herbs and other medicinal plants for treatment of diabetes in mammalian animal models is a very low priority and is costly, resulting in a gap in the drug development process. Fish are a vertebrate model organism that can bridge this gap. Recently, zebrafish have been proposed as a model for the regulation of glucose levels, by exposing adult and larval zebrafish to known anti-hyperglycemic compounds[4].

Ocimum tenuiflorum (*O. tenuiflorum*), a member of the family Lamiaceae (Labiatae), is a tropical herb, up to 18 inches tall, that grows into a low bush. The plant grows wild in Cuba but is also widely cultivated in gardens. The aqueous extract of *O. tenuiflorum* is used as an anti-hyperglycemic in Cuba[5]. In this study, we investigated the effect of *O. tenuiflorum* aqueous extract on level of blood glucose in tilapia [*Oreochromis niloticus (O. niloticus)*], as a new fish model for diabetes.

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2. Materials and methods

2.1. Fish

Male juvenile tilapia (*O. niloticus*) weighting (46.6 ± 4.8) g was supplied by Fisheries Department of Camagüey, Cuba. The animals were maintained in the laboratory at (27 ± 2)℃ in aerated water. Tilapia was kept throughout in a 14 h light: 10 h dark photoperiod and fed ad libitum with fish pellets containing 30% proteins (Malta-Cleyton, Mexico).

2.2. Inducing hyperglycemia in tilapia

Five groups were made with 7 animals in each. Glucose was added to the fish pond at 0, 25, 50, 75 and 100 g/L and fish were kept in this water for 30 min. Then the fish were passed to clean freshwater and blood samples were taken.

2.3. Hyperglycemic time course in tilapia

Two groups were made with 45 animals each. One group was used as control and to the other group hyperglycemia was induced by adding glucose to the fish pond at 50 g/L. Fish were pretreated for 30 min and blood samples were taken 0, 15, 30, 60, 90 and 120 min after the fish were passed to clean freshwater. Blood samples were also taken before the induction of hyperglycemia.

2.4. Plant and preparation of extract

O. tenuiflorum was collected from the organoponic "La Victoria", carretera central, Camagüey, Cuba; and was identified by Professor Jorge E. Gutiérrez of the Botany Department, University of Havana, Cuba. Voucher sample was deposited in the herbarium collection of the National Botanical Garden, Havana, Cuba (voucher specimen no. HAJB 87 101). An aqueous extract was prepared by boiling 120 g of the fresh leaves in approximately 1 L for 15 min. The extract was filtered, adjusted to a volume of one liter and used immediately. Part of the extract was placed in the oven (40℃) to detect the dry weight.

2.5. Evaluation of the O. tenuiflorum extract

Four groups were made with 7 animals each. One group without treatment was used as control and hyperglycemia was induced in the other three groups. After 30 min of incubation with glucose at 50 g/L the animals were passed to clean freshwater. Animals from the second group were injected with human insulin (Roche). The third group was incubated with *O. tenuiflorum* extract at 200 mg/L. The last group was incubated in freshwater without further treatment. After 1 h blood samples were taken from all animals.

2.6. O. tenuiflorum extract doses

Six groups were made with 7 animals each. One group without treatment was used as control and hyperglycemia was induced in the other fifth groups. After 30 min of incubation with glucose at 50 g/L the animals were passed to clean freshwater. The groups were treated with the doses 0, 40, 80, 200 and 400 mg/L of the *O. tenuiflorum* leaf extract, respectively. The last group was incubated in freshwater without further treatment. After 1 h blood samples were taken from all animals.

2.7. Blood samples and serum glucose measurements

About 150 μL of blood was removed from the caudal vein of each fish. The blood samples were incubated at 37 ℃ for 10 min, and serum were obtained by centrifuging at 2 000 *g* for 5 min. Glucose levels were estimated by commercially available glucose kits based on the glucose oxidase method (Quimefa®, Cuba).

2.8. Data analysis

All data were tested for normality and assessed by one-way analysis of variance (ANOVA). Differences were detected by Student-Newman-Keuls *Post-Hoc* Test.

3. Results

Adding glucose to fish pond water induced hyperglycemia in tilapia (*O. niloticus*). At 25 g/L of glucose in pond water increased the blood sugar concentration of tilapia by 27% compared to the control group. The blood sugar concentration in tilapia showed saturation when more than 50 g/L of glucose was added to the pond water. An average increase of 50% in blood sugar concentration compared to the control group was observed when 50 to 100 g/L glucose was added to the pond water (Figure 1).

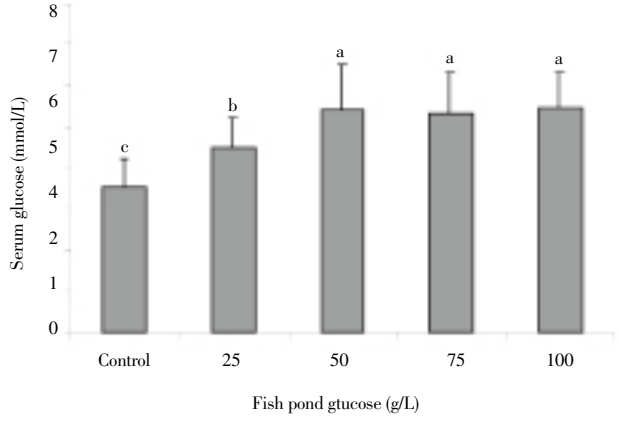

Figure 1. Induction of hyperglycemia in tilapia (*O. niloticus*) after glucose addition to the fish pond water.
Glucose was added to the fish pond at 0, 25, 50, 75 and 100 g/L, respectively. Thirty min later, animals were passed to clean freshwater and blood samples were taken. Different letters indicate statistical differences ($P<0.05$). T Bars indicate the SEM at each point ($n=7$).

Even 90 min after the induction of hyperglycemia in *O. niloticus*, the serum glucose was higher than the control group. The highest glucose blood value (6.76 ± 0.49) mmol/L was obtained 15 min after the beginning of the induction period. The blood glucose level remained high for 60 min after the fish incubation with glucose. The serum glucose decreased to (4.48 ± 0.24) mmol/L, but was still significantly higher than the control group (3.18 ± 0.29) mmol/L. Two hours after induction of hyperglycemia, the serum glucose dropped to values similar to those of the control group (Figure 2).

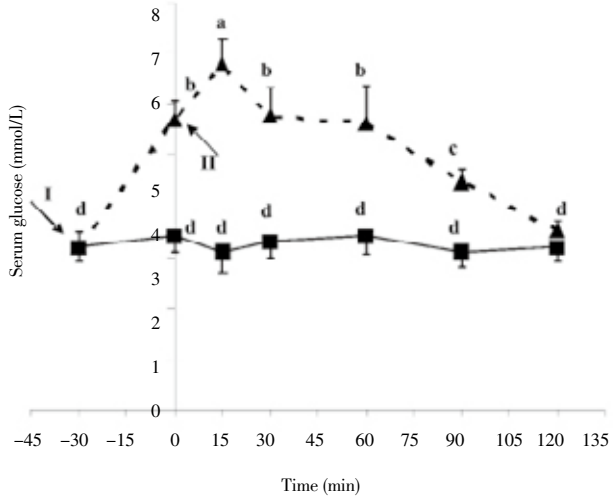

Figure 2. Time course of serum glucose in tilapia (*O. niloticus*) after induction of hyperglycemia.
Glucose was added to the fish pond at 50 g/L for the experimental groups; 30 min later the treated animals were passed to clean freshwater. Blood samples were taken before the glucose addition and at 0, 15, 30, 60 and 90 min after the induction of hyperglycemia. The control group didn't receive any treatment. Arrows indicate the beginning (I) and the end (II) of the induction period. Different letters indicate statistical differences (P<0.05). T Bars indicate the SEM at each point (n=5).

Administrating aqueous extract of *O. tenuiflorum* leaves showed a significant reduction of serum glucose levels in glucose-treated tilapia. The serum glucose values of tilapia treated with the plant extract (3.26 ± 0.62) mmol/L were similar to the control group (3.54 ± 0.55) mmol/L. The glucose serum in animals injected with human insulin also decreased to values (3.11 ± 0.58) mmol/L similar to the control group. The hyperglycemic group that did not receive further treatment had serum blood levels (5.39 ± 1.18) mmol/L significantly higher those of the control group.

The results showed that the leaf extracts of *O. tenuiflorum* possess significant blood sugar lowering potential in hyperglycemic tilapia model and were dose dependent. The serum glucose values of tilapia treated with 200 mg/L (3.25 ± 0.57) mmol/L and 400 mg/L (3.33 ± 0.55) mmol/L of plant extract were similar to the control group (3.09 ± 0.82) mmol/L. However, the hyperglycemic group that received 80 mg/L treatment had serum blood levels (4.51 ± 0.34) mmol/L significantly higher those of the control group, but lower than the hyperglycemic group that did not receive further treatment (6.33 ± 0.71) mmol/L. Moreover, the dose of 40 mg/L of *O. tenuiflorum* extract did not decrease the serum blood levels (6.26 ± 0.68) mmol/L in induced hyperglycemic tilapia.

4. Discussion

Diabetes mellitus is a serious chronic disease. Although oral anti-hyperglycemic agents and insulin are often successful in diabetes treatment[6], they have prominent side effects and fail to significantly alter the course of diabetic complications[7, 8]. Medicinal herbs are expected to have a similar degree of efficacy without the troublesome side effects associated with conventional drug treatment[9]. Ideally, preclinical *in vivo* experiments should be carried out before using herbal treatments[3, 10].

Various animal models have been developed to examine diabetes. Most models use primates, small mammals, mice or rats, in which diabetes has been chemically induced through the injection of streptozotocin or alloxan[11]. The chemicals used to induce insulin dependent diabetes have side effects, including kidney, lung and liver tumors (streptozotocin) or liver and kidney necrosis (alloxan), which illustrates the lack of specificity of these drugs and may affect blood glucose levels[12].

In the present paper, hyperglycemia was induced in tilapia by adding glucose to the fish pond water. This is in agreement with the induction of hyperglycemia in adult zebrafish that was proposed as a model to study diabetic retinopathy[13]. These authors found that zebrafish incubated in glucose solutions above 50 g/L suffered impairment or death within 4 h. To induce hyperglycemia in tilapia we incubated the fish for only 30 min and no fish death was observed.

Larval zebrafish may be an appropriate model for the examination of glucose metabolism, using phosphoenolpyruvate carboxykinase as an indicator of blood glucose levels[4]. Because it is larger, tilapia, has an advantage over zebrafish for studying glucose levels and organs. The data presented in this paper indicate that tilapia is a suitable model for testing medicinal herbs that have anti-hyperglycemic activity. The decision of which model of diabetes to use for any particular protocol is mainly influenced by local resources[3].

The decrease of serum glucose observed after the administration human insulin in tilapia (*O. niloticus*), is in agreement with the homology between fish and human insulins. Fish insulins have been demonstrated to be functional in mice and humans[14]. However, transgenic tilapia expressing "tilapia humanized insulin" have been examined as a way to avoid anti-insulin antibody production in human xenotransplantation[15].

In the present study, an aqueous leaf extract of *O. tenuiflorum* produced a significant fall in blood glucose levels of hyperglycemic tilapia. Different extracts of plants from the family Lamiaceae (Labiatae) have also been shown to have anti-hyperglycemia activity[16]. Intraperitoneal injection in

normal and diabetic rats of methanolic extract of *Ocimum gratissimum* leaves significantly reduced glucose plasma level by 56% and 68%, respectively[17]. Ethanolic extract of *Ocimum sanctum* leaves showed hypoglycemic effects (26%) in normal albino rabbits[2]. Administration of aqueous extract of *Ocimum canum* decreased fasting blood glucose levels in diabetic and non-diabetic C57BL/KsJ mice[18,19].

In this paper, it was observed that the leaf extracts of *O. tenuiflorum* possess significant dose-dependent blood sugar lowering activity in hyperglycemic tilapia. The fact that neither toxicity nor lethality was observed at any doses of the *O. tenuiflorum* extract explains the wide safety margin of the extracts within the doses range. This is in agreement with the findings ethanol and aqueous extracts of the leaves of *Ocimum suave* and *Ocimum lamiifolium* that showed antipyretic activities in mice[20].

This is the first report that demonstrates the serum-glucose lowering activity of the aqueous leaf extract of *O. tenuiflorum* in hyperglycemic fish. Further studies are necessary to reveal the mechanism of action of this extract in decreasing blood glucose level. The new tilapia model we are proposing may help to accelerate these studies and test plant extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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