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Evaluation of Biological Activity of Oil *Crocodylus moreletii* on Glucose levels Using a Diabetic Model

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Abstract: There are some reports which show that omega-3 fatty acids extracted from either animals or plants can be used to reduce glucose levels in diabetic patients; however, the results are very confusing; perhaps this phenomenon is due to the different protocols used. Analyzing these data, this study aimed to evaluate the biological activity of the oil extracted from *Crocodylus moreletii* at a dose of 100, 200, and 300 µl/kg using metformin glibenclamide and sodium oleate as controls in a diabetic rat model. The results showed that oil *Crocodylus moreletii* lowered glucose levels in a dose-dependent manner than untreated diabetic rats. Furthermore, the effects produced by *Crocodylus moreletii* oil and sodium oleate on glucose concentration were very similar. However, the biological activity exerted by glibenclamide and metformin on glucose levels was different from to effect induced by *Crocodylus moreletii* oil and sodium oleate. In conclusion, the biological activity of *Crocodylus moreletii* oil is interesting and could be considered a therapeutic alternative for the treatment of diabetes.

Keywords: *Crocodylus moreletii*; oil; glucose; metformin; glibenclamide.

1. Introduction

Diabetes mellitus is a risk factor for developing cardiovascular diseases [1-3]; it is important to mention that several drugs such as sulfonylureas [4], biguanides [5], α-glucosidase inhibitors [6], thiazolidinediones [7] have been used for the treatment of this clinical pathology. However, some of these drugs can produce side effects; for example, some studies have reported that the use of glibenclamide can be associated with severe hypoglycemia [8,9] and ventricular arrhythmia in diabetic men [10]. Besides, other reports indicate that metformin can be associated with lactic acidosis in diabetic patients [11]. Other data indicate that rosiglitazone could increase myocardial infarction risk in type II diabetic patients [12]. In search of therapeutic alternatives for the treatment of this pathology clinical, some diets have been developed which involve a diet based on fish and their derivatives that are a rich source of n-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid. For example, some epidemiological studies show that Alaskan Eskimos have a very high intake of fish, which has

been associated with a low prevalence of diabetes [13,14]. Besides, a study carried out in a Dutch population showed that fish consumption (8 g/24 hours) can induce changes in blood glucose levels [15]. Other reports suggest that consuming fish several times a week may decrease the risk of developing diabetes [16,17]. However, a prospective study in American adults (152,700 women and 42,504 men) showed that eating fish 5 servings of food a week does not reduce the development of diabetes mellitus; instead, higher intakes may modestly increase the incidence of this disease [18]. In addition, a study was conducted on 27 women with diabetes mellitus who consumed 3 g/day of fish oil (1.8 g/day of n-3 polyunsaturated fatty acids) for 2 months showed no changes in blood glucose levels [19]. Furthermore, a study carried out in 30 patients with diabetes mellitus (mean age 55 years) who consumed 930 mg eicosapentaenoic acid + 750 mg docosahexaenoic acid per day for 12 weeks showed that fasting glucose or insulin concentrations were not decreased [20]. All these data indicate great controversy about the biological activity exerted by omega-3 fatty acids on diabetes. In this way, this study evaluated the biological activity of oil (rich in omega-3 fatty acids) extracted from Crocodylus moreletii using a diabetic rat model.

2. Materials and Methods

2.1. General.

The reagents used in this research were acquired from Sigma-Aldrich Co., Ltd.

2.2. Sample collection.

The fatty portion of the crocodile meat (*Crocodylus moreletii*) was obtained at the Wotoch Aayin crocodile farm, located on Isla Arena in the municipality of Calkiní, Campeche, Mexico (Coordinates 20.6905, -90.4525).

2.3. Crocodile oil extraction.

In addition, the oil was extracted by a previously reported method [21]; the fat sample was cut into small pieces (5-10 cm in diameter) and then boiled at a temperature of 80 to 90 ^{o}C for 45 minutes. The crude oil was separated by centrifugation at 6000 rpm at a temperature of 4 ° C for 10 minutes. It was then stored at a temperature of 4 ^{o}C for later use.

2.4. Animals.

Male rats (200-250 g) were obtained from Pharmaco-Chemistry Laboratory, Faculty of Chemical Biological Sciences, University Autonomous of Campeche.

2.5. Induction of diabetes.

The animals were injected with alloxan monohydrate dissolved in sterile normal saline at a 150 mg/Kg body weight dose intraperitoneally [22].

2.6. Glucose analysis.

After 2 weeks, rats with moderate diabetes having glycosuria* (indicated by Benedict's qualitative test) and hyperglycemia (i.e., with blood glucose ≥ 200 mg/dL) were used for the experiment [22].

*Blood glucose was determined from tail blood with a rapid glucose analyzer (Accutrend Sensor Comfort; Roche, United States.) every 48 hours. The rats were divided into sixteen groups after the induction of diabetes. Six rats were used in each group (42 diabetic surviving rats and six normal rats).

2.7. Experimental design.

Group 1: Normal rats were given 2 ml of normal saline.
Group 2: Diabetic control rats given 2 ml of normal saline.
Group 3: Diabetic rats were given an aqueous solution of glibenclamide$^{\phi}$ (600 µg/kg body mass) daily with an intragastric tube for 30 days.
Group 4: Diabetic rats given an aqueous solution of metformin$^{\varpi}$ (350 mg/kg body mass) daily had an intragastric tube for 30 days.
Group 5: Diabetic rats were given *Crocodylus moreletii* oil (100 µl/kg) daily with an intragastric tube for 30 days.
Group 6: Diabetic rats were given *Crocodylus moreletii* oil (200 µl/kg) daily with an intragastric tube for 30 days.
Group 7: Diabetic rats were given *Crocodylus moreletii* oil (300 µl/kg) daily with an intragastric tube for 30 days.
Group 8: Diabetic rats given an aqueous solution of Sodium oleate§ (50 mg/ml) daily with an intragastric tube for 30 days.
$^{\phi,\varpi}$Dose administered of either glibenclamide or metformin were determinate using a previously reported method [21].
§Dose administered of Sodium oleate was based on a previously reported method [23]. In addition, the volume administered to rats via oral was 1 mL of each compound.

2.8. Statistical analysis.

The obtained values are expressed as average ± SE. The results were put under variance (ANOVA) analysis with the Bonferroni correction factor using the SPSS 12.0 program [24]. The differences were considered significant when p was equal to or smaller than 0.05.

3. Results and Discussion

3.1. Glucose levels.

There is great controversy about the biological activity of omega-3 fatty acids on diabetes; perhaps this phenomenon could be due to the different protocols used in each biological assay in either humans or animals [13-20,25-30]. Therefore, in this study, the biological activity of *Crocodylus moreletii* oil on glucose concentration in a diabetic animal model was evaluated. It is important to mention that diabetes in the animals studied was induced with alloxan; this compound can indirectly induce a reduction in insulin release through the elimination of β-cells from the islets of Langerhans, increasing glucose concentration [31]. The results showed that *Crocodylus moreletii* oil at dose of 100 µl/kg (332.00 to 128.00 mg/dl), 200 µl/kg (323.00 to 122.00 mg/dl) and 300 µl/kg (308.20 to 90.00 mg/dl; p = 0.05) decreased glucose concentration in a dose-dependent manner (Figure 1) compared with the diabetic rats without treatment (280 to 467 mg/dl).

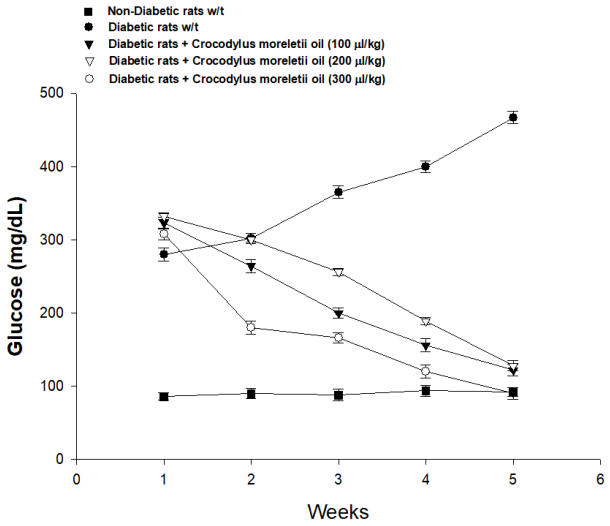

Figure 1. The biological activity produced by *Crocodylus moreletii* oil on glucose levels. The results showed that *Crocodylus moreletii* oil significantly decreased (p = 0.05) the glucose concentration in a dose-dependent manner compared with diabetic rats without treatment (control). Each point represents the mean ± S.E. of 6 experiments: wt = without treatment; S.E., standard error.

Analyzing these data, in this investigation, some alternative experiments were carried out to compare the effect exerted by *Crocodylus moreletii* oil with some drugs used for the treatment of diabetes, such as glibenclamide (potassium channels inhibitor) [32-34] and metformin (insulin receptor sensitivity) [35, 36]. The results showed that glibenclamide (332.00 to 84.00 mg/dl; p = 0.05), metformin (328.00 to 94.00 mg/dl; p = 0.06), and sodium oleate (330.00 to 102.00 mg/dL; p = 0.05) significantly diminished the blood glucose concentration in comparison with diabetic rats without treatment (Figure 2); however, the biological activity exerted by both glibenclamide and metformin on glucose levels was different to effect induced by *Crocodylus moreletii* oil at a dose of 300 µl/kg. These results suggest that the effect produced by *Crocodylus moreletii* oil on glucose is not due to the interaction with any target biomolecule for both glibenclamide and metformin drugs.

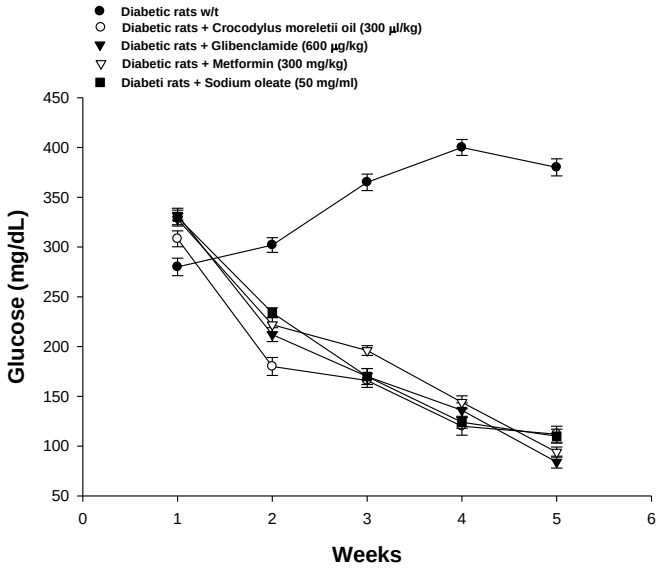

Figure 2. The effect produced by *Crocodylus moreletii* oil, glibenclamide metformin, and sodium oleate on glucose levels. The results showed that either glibenclamide or metformin significantly decreased (p = 0.05) the glucose concentration compared with diabetic rats without treatment. However, the biological activity exerted by *Crocodylus moreletii* oil on glucose levels was similar to sodium oleate. Each point represents the mean ± S.E. of 6 experiments: wt = without treatment; S.E., standard error.

Analyzing these data and other reports which indicate that *Crocodylus moreletii* oil, which is rich in oleate (the conjugate base of oleic acid) [37], can modulate either insulin or glucose levels [38, 39] In this way, the biological activity of oleate on glucose levels was evaluated using sodium oleate to compare it with the effect produced by *Crocodylus moreletii* oil. The results showed that sodium oleate decreased glucose concentration (330.00 to 102.00 mg/dl; p = 0.05) significantly compared with diabetic rats without treatment (Figure 2); however, this effect was similar to this *Crocodylus moreletii* oil. All these data suggest that the biological activity induced by Crocodylus moreletii oil on glucose levels could depend on oleate's effect, translating into a protective effect against diabetes.

3.1. Body mass levels.

Some alternative experiments were carried out to evaluate whether *Crocodylus moreletii* oil (100 to 300 µl/kg) could induce changes in the bodyweight of diabetic rats. The results showed that *Crocodylus moreletii* oil in doses of 100 µl/kg (256 to 263 g), 200 µl/kg (270 to 276 g), and 300 µl /kg (256 to 258 g) did not decrease body weight levels in diabetic rats (Figure 3). Analyzing these data, the biological activity of glibenclamide and metformin was also evaluated to compare with the effect exerted by *Crocodylus moreletii* oil at a dose of 300 µl/kg. The results showed that both metformin (260 to 220 g) and glibenclamide (250 to 226 g) slightly decreased body weight compared to either *Crocodylus moreletii* oil (256 to 276 g) or oleate sodium (250 to 272 g); nevertheless, this effect was significantly different (p = 0.05) from untreated diabetic rats (Figure 4). All these data indicate that both *Crocodylus moreletii* oil and oleate sodium did not decrease the bodyweight levels.

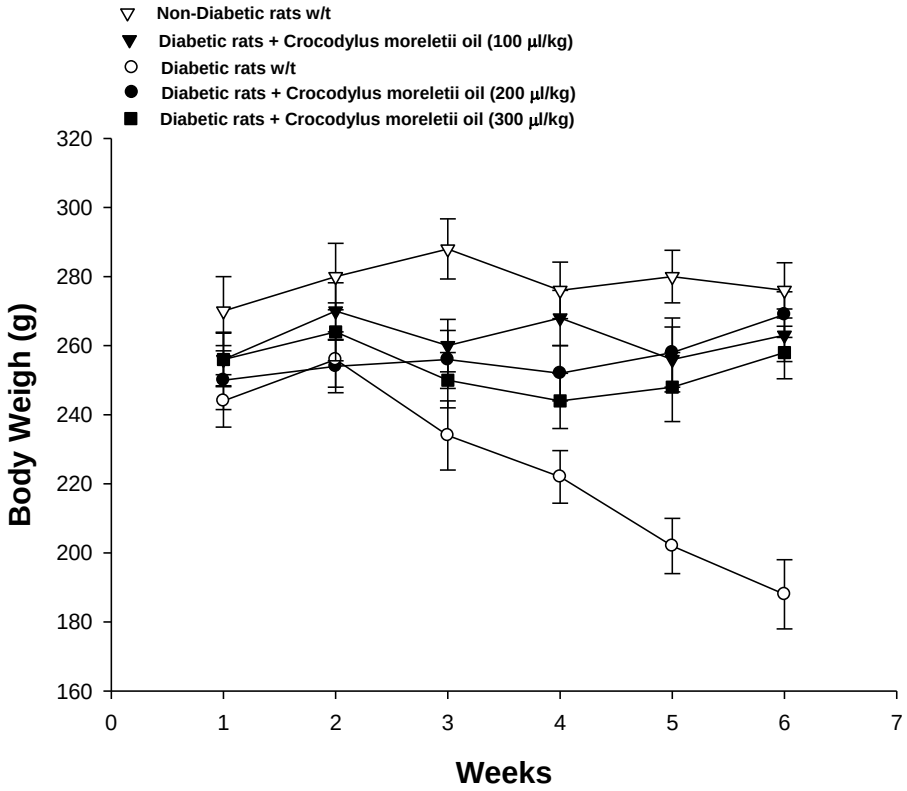

Figure 3. The effect produced by *Crocodylus moreletii* oil on body weight. The results showed that the *Crocodylus moreletii* oil in the different doses did not cause significant changes in the bodyweight of the diabetic rats. However, in untreated diabetic rats, it was significantly reduced (p = 0.05). Each point represents the mean ± S.E. of 6 experiments: wt = without treatment; S.E., standard error.

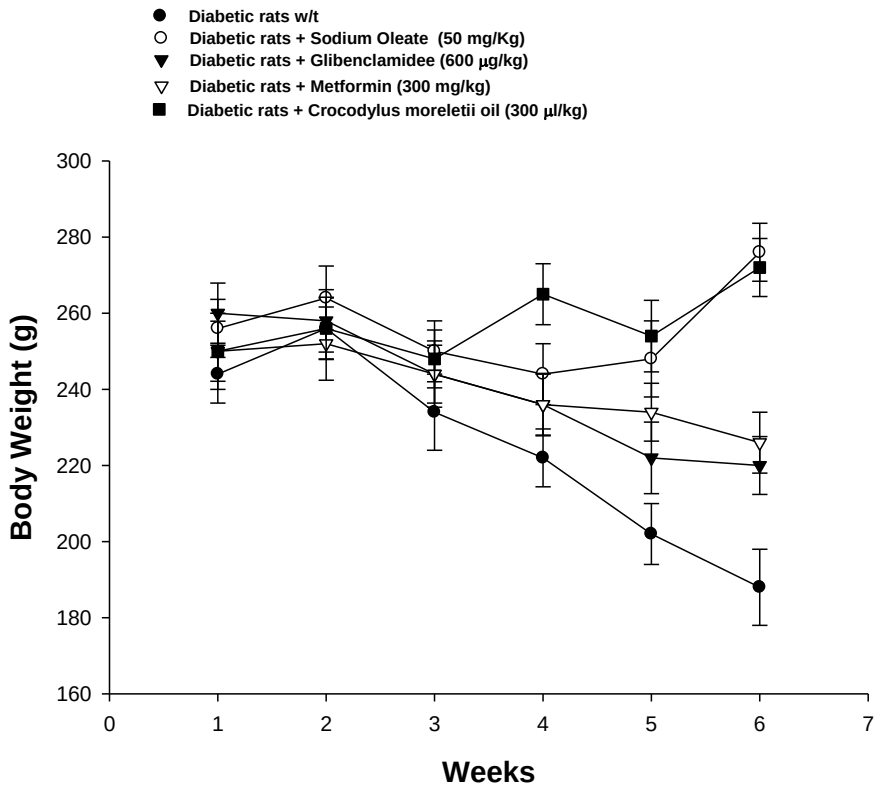

Figure 4. Biological activity exerted by *Crocodylus moreletii* oil, sodium oleate, metformin, and glibenclamide on body weight. Over time, the results showed that body weight decreased significantly ($p = 0.05$) in diabetic rats (without treatment, w/t). Furthermore, other data showed that both *Crocodylus moreletii* oil and sodium oleate did not cause significant changes in the body weight of diabetic rats. However, in the presence of metformin or glibenclamide, the body weight of diabetic rats was slightly reduced. Each point represents the mean ± S.E. of 6 experiments: wt = without treatment; S.E., standard error.

4. Conclusions

This study suggests that the effect exerted by *Crocodylus moreletii* oil on glucose levels are associated with biological activity induced by oleate, which is required to lower glucose levels. Therefore, the biological activity of *Crocodylus moreletii* oil is interesting and could be considered a therapeutic alternative for the treatment of diabetes.

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Conflicts of Interest

We declare that this manuscript does not have any conflict of financial interests (political, personal, religious, ideological, academic, intellectual, commercial, or otherwise) for its publication.

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