ORIGINAL RESEARCH

Bixa orellana (annatto) exerts a sustained hypoglycemic effect in experimental diabetes mellitus in rats

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OBJECTIVE: Bixa orellana (annatto) is a natural pigment and food colorant that has been used for a variety of therapeutic purposes. It has been suggested that annatto could have the property of reducing blood glucose levels. However, most previous studies have demonstrated a hypoglycemic effect in non-diabetic animals. We evaluated the impact of annatto on blood glucose levels in an experimental model of diabetes mellitus.

METHOD: Male Wistar rats were made diabetic by a single dose of60 mg/kg streptozotocin, injected intraperitoneally. One group of diabetic animals was treated with annatto, 540 mg/kg, administered via gavage.

RESULTS: Annatto treated diabetic animals showed significantly lower blood sugar levels than untreated diabetics. The blood glucose curve showed that the hypoglycemic effect of annatto lasted for the twelve hours of evaluation. However, the most significant reduction on blood glucose took place two hours after drug administration. Annatto did not prevent the weight loss that is characteristic of decompensated diabetes mellitus.

CONCLUSION: Annatto has a hypoglycemic property in rats with severe diabetes mellitus. Such an effect had a relatively long duration, but is more pronounced two hours after drug administration.

KEYWORDS: Bixa orellana; Diabetes Mellitus; Rats.

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INTRODUCTION

In the last decades, several plants have been used for a variety of therapeutic purposes. *Bixa orellana L*, commonly known in Brazil as Urucum, is a shrub native to the tropical zone of the Americas. Its seeds have been used for centuries by American people as the source of annatto, a natural pigment and food colorant.[1,2] Bixin is a carotenoid that constitutes 80% of the pigments found in *Bixa orellana*. As for its medical use, several biological properties have been described. The ethanol extract from its leaves presents antibiotic activity against Gram-positive microorganisms and *Candida albicans*.[3,4] The water extract from the seeds has antihypertensive properties and the hydroalcoholic extract from the fruit triggers analgesic and anti-inflammatory activities in mice.[5] Furthermore it was also demonstrated that bixin has lipo-reducing properties.[6]

More recently, it was suggested that bixin could have the property of reducing blood glucose levels.[7] However, most previous studies have demonstrated the reduction of blood sugar levels in non-diabetic animals. In fact, there have been few reports of the effects of bixin on experimental models of diabetes mellitus.[8,9,10] The present study aims at evaluatingthe impact of annatto on blood glucose levels in an experimental model of diabetes mellitus.

METHODS

Male Wistar rats were used, weighing approximately 240g, provided by the vivarium of Experimental Surgery of the State University of Health Sciences of Alagoas (Universidade Estadual de Ciências da Saúde de Alagoas). All the animals were kept under aseptic conditions in cycles of 12/12 hours light/darkness, at a temperature of approximately 22°C. They were fed standard rat chow (Labina®, Agribands Purina do Brasil Ltda, Paulínia, SP, Brazil) and water *ad libitum*. The project submitted to and gained approval by the Institutional Committee of Ethics in Research.

Diabetes mellitus was induced through the intraperitoneal injection of streptozotocin (SZT – SIGMA), in a single dose of 60 mg/kg body weight, diluted in a citrate buffer, after anesthesia with sodium thiopental.[11]

The animals were divided into three experimental groups, with eight animals per group:

CN – non-diabetic control group, not treated with annatto
DM – diabetic group, not treated with annatto.
DMa – diabetic group treated with annatto, with a daily dose of 540 mg/kg, administered via gavage.

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In a preliminary experiment the dose of 540 mg $*$ kg^{-1} $*$ day^{-1} annatto was found to be the maximum dose that rats would tolerate without growth stunting or deterioration of their general condition. All animals had free access to tap water and standard chow (0.5% Na, 22% protein). None of the animals was treated with insulin throughout the entire period of study. During the twelve-day-long experiment, we performed daily measurements of the animals' body weight and blood sugar level (through a digital glucosimeter - One Touch® Ultra™/Lifescan).

The experiment was divided in two phases. In this first phase, which lasted ten days, the animals treated with annatto received the drug between 4:00 and 5:00 P.M.; blood sugar levels were measured in the morning of the following day. In the second phase, on the eleventh day of treatment, annatto was administered in the morning and a blood glucose curb was drawn, with measurements of blood sugar level before drug administration (zero time), then at two, six and twelve hours after annatto ingestion. At 8:00 A.M. of the following day, blood glucose level was measured for the last time (12th day).

Graph-Pad Prism (version 5.0) was used for the statistical analysis. The results were presented with means ± standard deviation. The comparison between groups was made by analysis of variance (ANOVA). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Diabetic animals exhibited significantly lower body weights than controls (DM = 255 ± 15 g, DMa 262 ± 17 g, $p < 0.05$ *versus* CN = 276 ± 21 g – mean ± standard deviation). However, there was no significant difference of body weight levels between the DM and DMa (Figure 1). The blood sugar levels of diabetic animals were considerably higher than those of the control group (Figure 2). However, the diabetic animals treated with annatto showed significantly lower blood sugar levels than untreated diabetic animals (DM = 507 ± 48 mg/dL, $p < 0.05$ versus DMa = 423 ± 18 mg/dL). But there was a highly significant difference of blood sugar levels between the control group and the annatto treated diabetic animals (DMa = 423 ± 18 mg/dL, $p < 0.01$ versus CN = 100 ± 2 mg/dL).

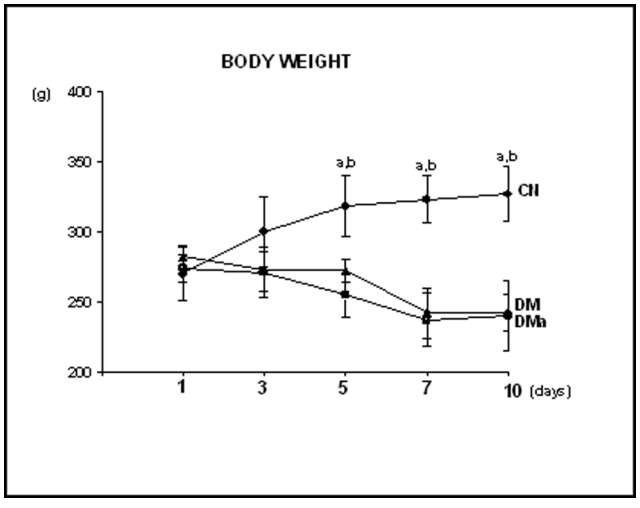

Figure 1 - Evaluation of the body weight during experiments. a: $p < 0.05$ CN versus DM and b: $p < 0.05$ CN versus DMa. CN: non diabetic untreated controls; DM: untreated diabetic rats; DMa: annatto treated diabetic animals.

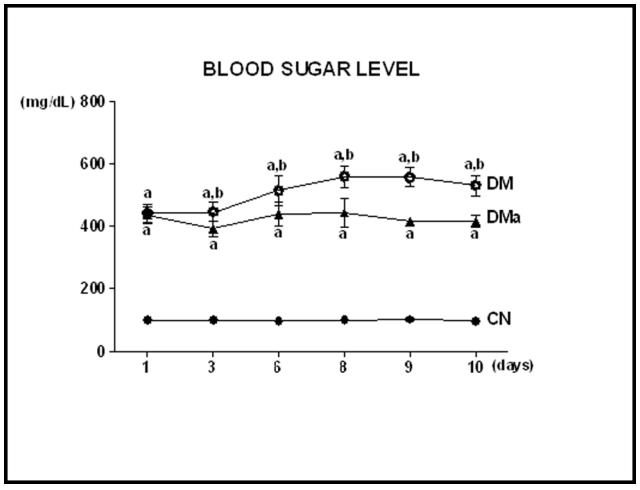

Figure 2 - Evaluation of the blood sugar levels in the experiment's first phase. a: $p < 0.05$ DM or DMa versus CN and b: $p < 0.05$ DM versus DMa. Abbreviations as in Figure 1.

The blood glucose curve performed on day 11 (Figure 3), demonstrates that the blood-glucose-reducing effect of annatto persisted for the twelve hours of evaluation. However, the most significant reduction of the blood sugar levels occurred two hours after drug administration (CN = 100 ± 2 mg/dL; DM = 529 ± 32 mg/dL and DMa = 380 ± 43 mg/dL).

DISCUSSION

In the present study, we observed that the extract of urucum (annatto), orally administered, had blood-glucose-reducing effects on diabetic animals. These findings are in accordance with those of previous investigations with non-diabetic and diabetic animals.[8-10]

The dynamics behind the blood-sugar-reducing effects of annatto are not yet completely understood. Russel *et al* demonstrated blood-sugar-reducing properties of annatto in

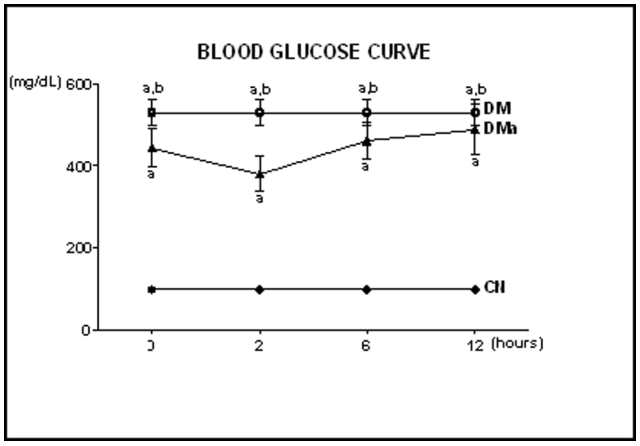

Figure 3 - Evaluation of the blood glucose curve on the 10th day of experiments. a: $p < 0.05$ DM and DMa versus CN and b: $p < 0.05$ DM versus DMa. Abbreviations as in Figure 1.

non-diabetic dogs.[7] That study evidenced a significant increase of serum insulin levels during the period of highest reduction of blood sugar levels, as well as an increase of affinity between insulin and its receptor. More recently, the same authors, using a model of streptozotocin-induced diabetes in dogs, observed that the reduction of blood sugar levels took place after annatto administration, but this effect was not accompanied by an increase of C-peptide levels.[10] This finding suggests that the blood-sugar-reducing effect does not happen because of a higher production of insulin, but rather as a consequence of increased peripheral use. This hypothesis could be strengthened if experiments were performed using models of type 2 diabetes mellitus, whose main characteristic is its peripheral resistance to insulin. Such models would require genetic manipulation. The relatively poor reduction of blood sugar levels in our study may have been determined, among other factors, by the diabetes mellitus model we used. Streptozotocin infusion via intraperitoneal injection induces a model of type 1 diabetes mellitus that is more aggressive than non-insulin dependent genetic models.[11,12] The animals thus develop very high blood sugar levels (above 500 mg/dl) and significant weight loss when not treated.[11] The findings in the second phase of the study (blood glucose curve) revealed that the strongest blood-sugar-reducing effect of annatto occurred after two hours following drug administration, but remained significant for the twelve hours of analysis. These data are similar to those of a previous study in dogs, which observed the strongest blood-sugar-reducing effect one hour after drug administration.[10] Unlike previous studies, we performed a more prolonged glycemic curve and observed a sustained hypoglycemic effect. Additionally, and because of the long interval between annatto administration (4:00 - 5:00 P.M.) and the measurement of blood sugar levels (8:00 A.M. on the following day), we conclude that the blood-sugar-reducing effect of annatto is even longer when orally administered, lasting for at least sixteen hours.

CONCLUSIONS

Annatto exhibited a blood-sugar-reducing effect on rats in the model of streptozotocin-induced diabetes mellitus. However, this effect was small and did not prevent the weight loss that is characteristic of decompensated diabetes mellitus. The effect had a relatively long duration (sixteen hours), which was more pronounced two hours after drug administration. However, new studies are necessary to evaluate the drug's effect on less aggressive models of diabetes mellitus.

RESUMO

OBJETIVO: Bixa Orellana (annatto) é um pigmento natural e corante alimentar, que tem sido proposto para uma variedade de usos terapêuticos. Foi sugerido que o annatto tem a propriedade de reduzir a glicemia. No entanto, a maioria dos estudos anteriores demonstrou um efeito hipoglicêmico em animais não diabéticos. Avaliamos o impacto do annatto sobre níveis sanguíneos de glicose num modelo experimental de diabetes mellitus.

MÉTODO: Ratos Wistar machos foram tornados diabéticos por uma única dose, 60 mg / kg de estreptozotocina, injetada por via intraperitoneal. Um grupo de animais diabéticos foi tratado com annatto, 540 mg / kg, administrado por meio de sonda gástrica:

RESULTADOS: Os animais diabéticos tratados com annatto exibiram níveis de glicemia significativamente mais baixos do que os diabéticos não tratados. Uma curva glicêmica revelou que o efeito hipoglicêmico do annatto perdurou durante as 12 horas de avaliação. No entanto, a redução glicêmica mais significativa ocorreu duas horas após a administração da droga. O annatto não impediu a perda de peso que é característica do diabetes mellitus descompensado.

CONCLUSÃO: O annatto apresenta propriedade hipoglicemiante em ratos com diabetes mellitus pós estreptozotocina. Este efeito teve uma duração relativamente longa, mas foi mais pronunciado duas horas após a administração da droga.

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