

ABSTRACT

AN IMPROVED METHOD OF ISOLATION OF TRANS-BIXIN, FROM ANNATTO (BIXA ORELLANA L.) SEEDS AND A STUDY OF ITS EFFECT ON INSULIN RECEPTORS AND GLUCOSE TRANSPORT IN THE DOG.

In an effort to assess the efficacy of the use of annatto extracts as a cure for diabetes, the *trans* isomer of bixin was isolated from the extract and characterized as a hyperglycemic and bioactive agent in clinically normal dogs. Bixin, a pigment used for food coloring is known to have hyperglycaemic effects. It was extracted using supercritical carbon dioxide, from seeds of annatto (*Bixa orellana* L.). Trans-bixin, the major pigment in annatto was quantified by HPLC. A combination of static and dynamic mode of extraction with acetonitrile (0.05% TFA) as modifier at 600 atm and 40°C was found to be the best set of SFE conditions studied. This method afforded 2.70 mg bixin gram⁻¹ dry weight of annatto seeds.

Oral glucose tolerance tests (OGTT) indicated also that dogs fed *trans*-bixin after an overnight fast, had an abnormally low tolerance to glucose, and this resulted in persistent hyperglycemia. A time course assay of insulin binding to isolated erythrocytes and leucocytes taken from dogs fed *trans*-bixin, was done on samples obtained during the OGTT. Preliminary results from Scatchard analyses suggest a decrease in the percentage of insulin bound to its receptor. This decrease was secondary to a significant reduction in the number of binding sites per erythrocyte ($P < 0.05$) one hour after *trans*-bixin was given via a gastric

tube and two hours later ($P < 0.01$). For leucocytes, the administration of *trans*-bixin decreased the number of insulin binding sites when tested one hour then two hours later. The affinity constants were significantly different for both erythrocytes and leucocytes when compared to the control. Adipose tissue biopsies from a separate population of starved, normal dogs, fed various dose-related amounts of *trans*-bixin, were also assayed using 3-O- methylglucose to measure glucose transport. The results of these studies led to the conclusion that the glucose transporters in mongrel dogs were susceptible to a dose related amount of *trans*-bixin two hours after a dose ($P < 0.05$). There was no significant difference at one hour when compared to the control. These investigations suggest that the mechanism of *trans*-bixin's diabetogenicity in the dog is a result of its impairment of peripheral glucose utilisation. It is thought that bixin like other fatty acids of similar structure is taken up by the cells and oxidised intracellularly. As a result of this, glycolysis and glucose uptake are inhibited and glucose levels increase. The combination of these factors eventually leads to antagonism of insulin action and hence the observed decrease in all the measurable parameters of insulin binding.