Hypoglycemic activity of a fucan from *Himanthalia elongata*

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INTRODUCTION

In previous report[1,2] a variety of assays were carried out in order to establish the hypoglycemic effects of polysaccharide extracts from the brown algae *Himanthalia elongata*. Crude polysaccharide and protein extracts of the seaweed lowered blood glucose levels in normal and alloxan-diabetic animals. In this work an active fucan was isolated from a calcium chloride fraction and assayed for its antidiabetic properties in rabbits.

MATERIALS AND METHODS

PREPARATION OF THE SEAWEED AND ISOLATION PROCEDURES

Himanthalia elongata (L.) S.F. Gray (*Himanthaliaceæ*) was collected on Porto Nadelas beach (La Coruña, Spain) by hand-picking at low tide. Fresh material was washed with tap water, cut into pieces and dried. The powdered dried algae was previously extracted with methanol and the algae residue extracted again with 2% calcium chloride[3]. The extractive solution was then dialyzed against water and the concentrate dialysate poured into excess of ethanol, and a solution of the resulting precipitate in water was freeze-dried. An aliquot was then dissolved in water and applied to the top of a DEAE-cellulose column (48 x 2,5 cm). The column was eluted with water and then with an increasing gradient of 0.1 M potassium chloride. 20 ml fractions were collected and monitored for carbohydrate[4]. A polysaccharide was obtained at about 0.3 M KCl (Fig. 1).

GENERAL METHODS

The percentage yield is based on the dry weight of the seaweed.

The carbohydrate content was determined as per DUBOIS *et al.*[5].

In order to analyse the monosaccharide residues which constitute the polysaccharide a cellulose thin layer chromatography was carried out with the products of acid hydrolysis of the polysaccharide (standard of fucose was analyzed by the same chromatographic method).

Sulphate content was determined as per DOGSON[6].

Glucose levels in serum were determined by the hexokinase method in a Seralyzer reflectance photomer (Ames).

Serum insulin was evaluated by radio-immuno-assay using a commercially available Kit (ICN biomedicals).

Statistical analysis was made with Student's t test.

BIOLOGICAL ASSAYS

Effect on serum glucose and insulin levels in normal rabbits.

Normal male N.Z. rabbits were dosed intravenously with 1.25, 2.5 and 5 mg/kg of polysaccharide and blood glucose levels determined at 0, 1, 3, 6 and 8 hours. Because maximal hypoglycemic activity was observed 8 hours after administration, serum insulin levels were also measured at this hour.

Effect on alloxan-diabetic rabbits

Diabetic rabbits were obtained by *i.v.* injection of 150 mg/kg of alloxan monohydrate dissolved in saline[7]. Animals with fasting glycemia of 300% or more were used. Initial blood glucose levels were determined to the fasted rabbits and a dose of 2,5 mg/kg was then administered *i.v.* Glycemia were measured again at the 8th hour.

RESULTS AND DISCUSSION

Percentage yield, carbohydrate and sulphate contens are given in Table 1. Chromatographic examination of hydrolysed polysaccharide gave only fucose.

Table 1
Fucan extracted by calcium chloride.

Percentage	Fucan
Yield	34.66
Carbohydrates	43.86
Sulphate	26.5

This fucan significantly lowered glycemia in normal rabbits 8 hours after *i.v.* administration at dose of 2.5 mg/kg (27% reduction) (Fig. 1).

However, insulin levels were not significantly modified 8 hours after administration (Fig. 2). When active dose was administered

to alloxan-diabetic animals, blood glucose levels were reduced to about 25% at the 8th hour (Fig. 3). Although glycemia was reduced in normal and diabetic animals, the belated hypoglycemic effect is not apparently due to an insulin release from pancreas. New assays are being carried out with this fucan in an attemp to define the mechanism of action of the hypoglycemic activity of this seaweed.

Fig. 1

Effects of fucan on serum glucose levels of normoglycemic rabbits. Each point represents the mean ±S.E.M. of 6-8 rabbits. Significantly different from control: * $p < 0.05$; ** $p < 0.01$.

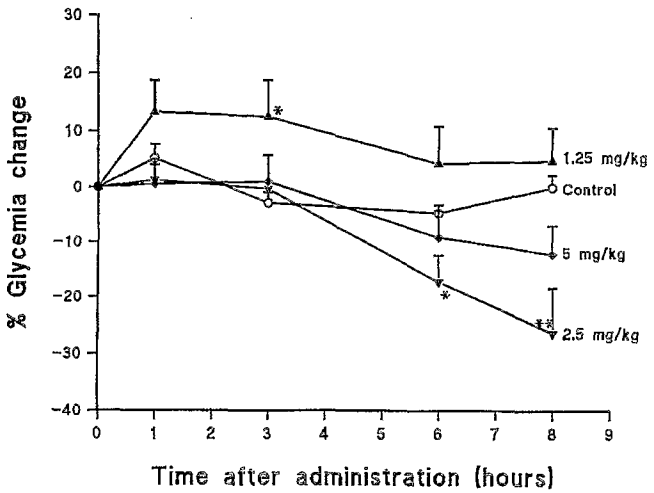


Fig. 2

Effects of fucan on serum levels insulin of normoglycemic rabbits. Each point represents the mean ±S.E.M. of 6-12 rabbits.

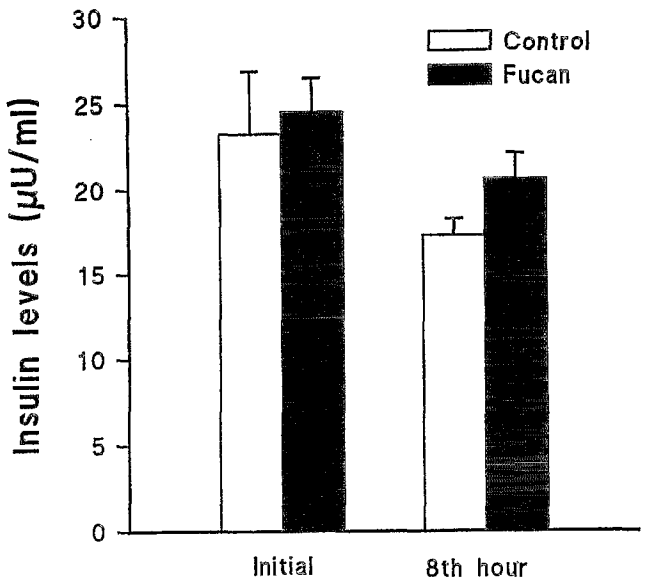
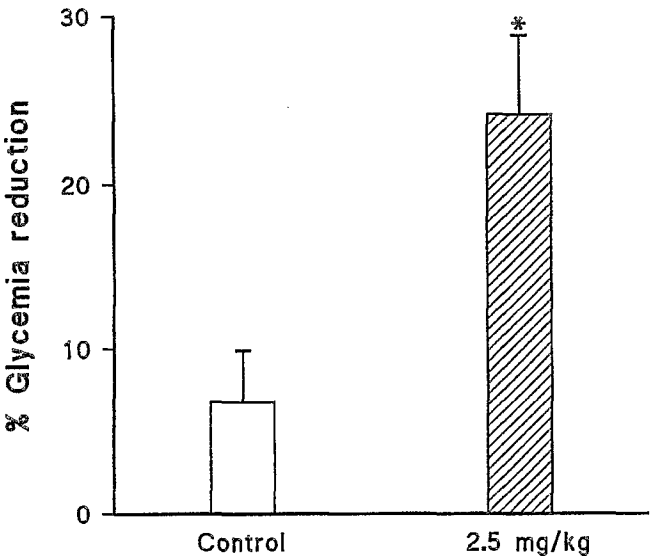


Fig. 3

Effects of fucan on glucose levels of diabetic rabbits 8 hours after administration. Each point represents the mean ±S.E.M. of 5 rabbits. Significantly different from control: * $p < 0.05$.



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