Study of several polysaccharide fractions from *Himanthalia elongata* on normoglycemic rabbits

LAMELA M., ANCA J.M., VAZQUEZ-FREIRE M.J., GATO A. AND CALLEJA J.M.
Department of Pharmacology, Pharmacy and Pharmaceutical Technology,
Faculty of Pharmacy. University of Santiago de Compostela.
E-15706 Santiago de Compostela, Spain.

INTRODUCTION

Several *Sargassum, Cystoseira* (*Phaeophyceæ*), *Corallina* and *Pterocladia* (*Rhodophyceæ*) species have been shown to possess hypoglycemiant properties[1]. A preliminar study was then carried out in our laboratory in order to determine the hypoglycemic activity of certain seaweeds. A crude polysaccharide extract from *Himanthalia elongata* significantly lowered the blood glucose levels in normal and alloxan-diabetic rabbits[2]. In this work several polysaccharide fractions were obtained and their effects on normoglycemic animals evaluated.

MATERIALS AND METHODS

SEAWEED COLLECTION AND PREPARATION OF EXTRACTIVE SOLUTIONS

Himanthalia elongata (L.) S.F. Gray (*Himanthaliaceæ*) was hand picked at low tide on Porto Nadelas beach (La Coruña, Spain). Specimen was authenticated by the Plant Biology Department of the University of Santiago de Compostela, Spain.

The fresh algal fronds were washed with tap water, cut into pieces and dried in a forced-air oven at 40 °C. The algae was then reduced to a fine powder and extracted with methanol in a soxhlet apparatus. After this operation, a sequential extraction was carried out as per Mian and Percival[3]. The air-dried seaweed was extracted with the following reagents:

I. 2% Aqueous calcium chloride for 4 h (twice at room temperature and once at 70 °C), with constant stirring.

II. Dilute hydrochloric acid (pH 2) for 4 h at 70 °C (four times).

III. 3% Aqueous sodium carbonate for 4 h at 50 °C (four times).

IV. Ammonium oxalate-oxalic acid (0.25% with respect to each, pH 2.8) for 6 h at 70 °C (two times).

All extractive solutions were filtered. The filtrates were concentrated in vacuo to a short volume and dialyzed against distilled water. The non-dialyzed portions were then freeze-dried and used to assay. The four fractions were denominated FI, FII, FIII and FIV respectively.

EFFECT ON NORMOGLYCEMIC ANIMALS

Normal male N.Z. rabbits weighing approximately 2 kg were used. All assays were performed on animals fasted for 20 h with water allowed *ad libitum*. Extractive solutions were administered intravenously via the ear vein and blood samples withdrawn by puncture from the contralateral ear vein. The concentration of glucose in serum samples was measured in a Seralyzer reflectance photometer (Ames, Miles Laboratories) using Seralyzer reagent strips (Hexokinase method). Initial glycemia was determined and extractive solution administered. Blood samples were taken 1, 3, 6 and 8 hours after administration and percentage variations of glycemia with respect to the initial level calculated[4]. Animals dosed with distilled water were used as controls.

GENERAL METHODS

Percentage yields of fractions are based on the dry weight of the algae.

The carbohydrate content of polysaccharide solutions was determined by the phenol-sulfuric acid method[5].

Statistical analysis of results was evalutated using Student's t test[6]. Data are given as means ±S.E.M. p values < 0.05 were taken to indicate significance.

RESULTS AND DISCUSSION

The yields of the secuential extraction corresponding to fractions and respective percentages of carbohydrate content are shown in Table 1.

Table 1
Polysaccharides fraction I, II, III and IV.

Fraction	% Yield	% Carbohydrates
FI	4.1	12.9
FII	5.1	30.9
FIII	3.0	36.4
FIV	1.1	9.5

Fig. 1.
Effects of Fraction I on serum glucose levels of normoglycemic rabbits. Each point represents the mean ±S.E.M. of 6 rabbits. Significantly different from control: * $p < 0.05$; ** $p < 0.01$.

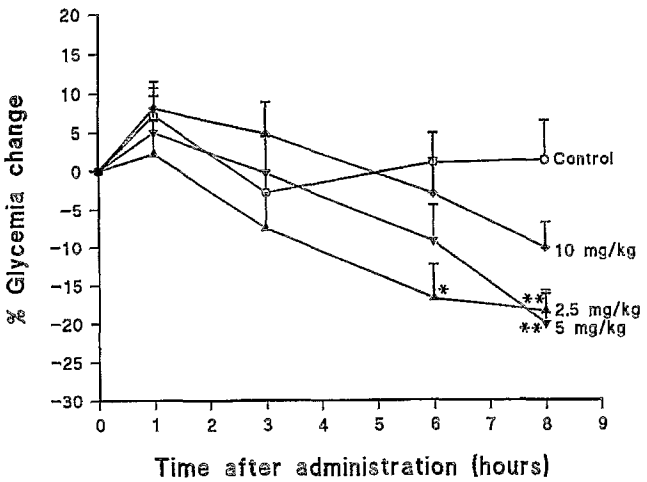


Fig. 1 shows the effect of assayed doses of calcium fraction (FI) on blood glucose levels. 2, 5 and 5 mg/kg caused a significant 18% and 20% drops in glycemia in normal animals, respectively, 8 hours after adminstration. Dose of 10 mg/kg was achieved 10% reduction at the same hour but this effect was not significant. No dose-response relationship was found.

Fig. 2.
Effects of Fraction II on serum glucose levels of normoglycemic rabbits. Each point represents the mean ±S.E.M. of 6 rabbits. Significantly different from control: * $p < 0.05$.

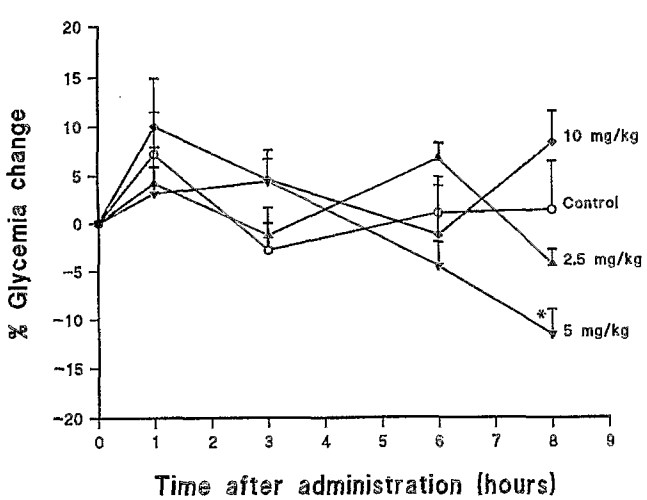


Fig. 2 illustrates the effect of FII. Only a dose of 5 mg/kg lowered glycemia 8 hours after administration though in this case a significant 11% drop was observed.

Fig. 3.
Effects of Fraction III on serum glucose levels of normoglycemic rabbits. Each point represents the mean ±S.E.M. of 6 rabbits.

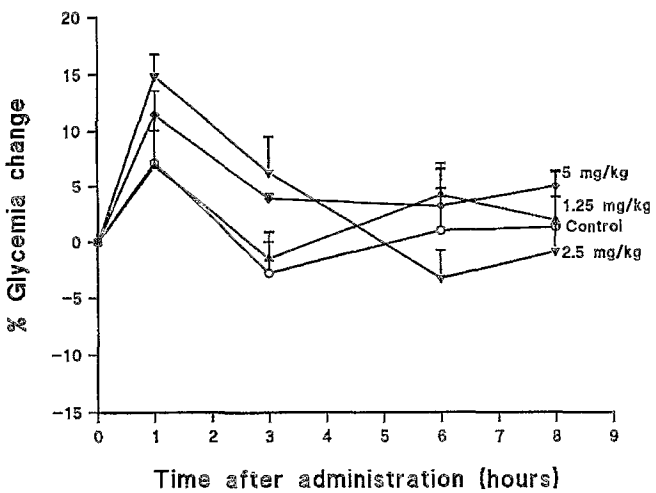
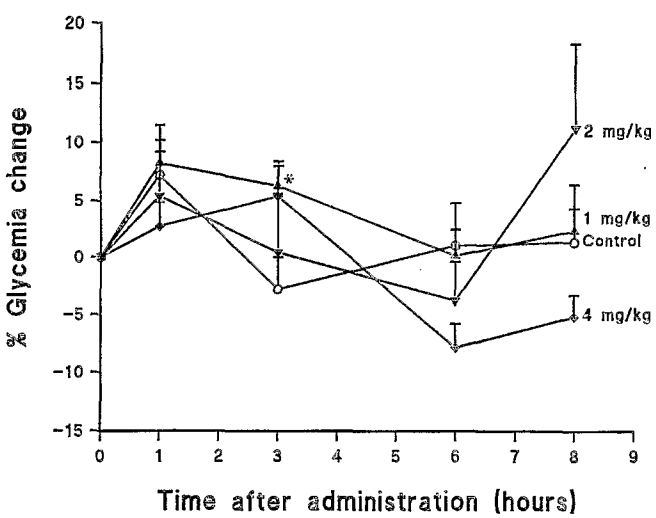


Fig. 4.
Effects of Fraction IV on serum glucose levels of normoglycemic rabbits. Each point represents the mean ±S.E.M. of 6 rabbits. Significantly different from control: * $p < 0.05$.



Neither FIII nor FIV from *H. elongata* produced any significant change in glycemia on the 8th hour though slight reductions were noted when a dose of 4 mg/kg of FIV was administered (Figs. 3 and 4).

We can conclude that the hypoglycemiant properties of *H. elongata* are basically due to the presence of the active principle in Fraction I.

REFERENCES

1. BÉZANGER-BEAUQUESNE L., 1982, *Plantes Medicinales et Phytothérapie,* 16, 73-95.

2. LAMELA M., ANCA J., VILLAR R., OTERO J. and CALLEJA J.M., 1989, *Journal of Ethnopharmacology,* 27, 35-43.

3. JABBAR MIAN A. and PERCIVAL E., 1973, *Carbohydrates Research* 26, 133-146. 1973.

4. LAMELA M., CADAVID I., GATO A. and CALLEJA J.M., 1985, *Journal of Ethnopharmacology,* 14, 83-91.

5. DUBOIS M., GILLES K.A., HAMILTON J.K., REBER P.A. and SMITH F., 1956, *Analytical Chemistry* 28, 350-356.

6. TALLARIDA R.J. and MURRAY R.B., 1986, *Manual of pharmacologic calculations with computer programs,* 2nd Ed., Springer-Verlagl., N.Y.