

## Neuropharmacological activity of *Cystoseira usneoides*

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### INTRODUCTION

In recent years, many studies have been carried out on marine organisms in order to discover possible pharmacological activities<sup>1</sup>. In this way, several marine seaweeds have been investigated in our laboratory for their effects on glycemia and diabetes<sup>2,3</sup>. When an extractive solution from *Cystoseira usneoides* was assayed, no effect on blood glucose levels was noted, but a notorious quiescent behaviour a few minutes after administration was observed. A preliminary study was then carried out in order to investigate the possible neuropharmacological effects of *C. usneoides*.

### MATERIALS AND METHODS

#### PREPARATION OF THE SEAWEED EXTRACT

*Cystoseira usneoides* (L.) Roberts (*Cystoseiraceae*) was hand-picked on Porto Nadelas beach (La Coruña, Spain) at low tide. The fresh material was washed, cut into pieces and dried at 50 °C. The powdered material was extracted with boiling methanol (4 vol.) three times. The residual seaweed was then dried and extracted with 2% aqueous calcium chloride for 4 hours with stirring, twice at room temperature and once at 70 °C<sup>4</sup>. Filtered solutions were combined and concentrated *in vacuo* to a short volume. This solution was then poured into excess of ethanol and the resulting precipitate dissolved in water and dialyzed against distilled water. The non-dialyzed portion was finally freeze-dried (percentage yield respect to initial dried algæ = 4) and assayed at doses of 6.25, 12.5 and 25 mg/kg.

#### Animals

All experiments were carried out using Male CD-1 mice (25 ± 3 g), except in the test of sodium pentobarbital-induced hypnosis, where females were employed. Animals were maintained, 3 days prior to assay and during its realization, in a soundless, temperature-controlled room (22 ± 1 °C), with a dark-light cycle of 12 hours.

Drugs and test fraction were dissolved in distilled water and dosed intraperitoneally (0.1 ml/10 g body weight).

Control animals were given distilled water.

### BIOLOGICAL ASSAYS

#### Spontaneous locomotor activity

The effect of *Cystoseira usneoides* on the locomotor activity of groups of 4 mice was registered, immediately following the administration, with an activity cage (Panlab), equipped with an electromagnetic field; the energy of this field is altered by any animal motion, and each change is recorded as a locomotion count<sup>5</sup>. Spontaneous locomotor activity was cumulatively measured over 1 h at 10 min intervals.

#### Exploratory behaviour

Exploratory behaviour was tested in the holeboard apparatus: a black board with 16 holes, symmetrically distributed in 4 rows. The number of head-dips was cumulative measured by a photobeam system placed under the box<sup>6</sup>. Recording of exploratory activity started immediately after administration of vehicle or extract to groups of 4 mice and went on at 10 min intervals up to 60 min.

#### Body temperature

Rectal temperature was recorded to groups of 8-14 mice, before and after administration of control or extract at predetermined time intervals, using a thermo-electric probe linked to a digital thermometer (Panlab).

#### Effect on pentobarbital-induced hypnosis

Mice were dosed with distilled water (control) or *Cystoseira usneoides* extract. 30 min later, they were given *i.p.* 55 mg/kg of sodium pentobarbital. The sleeping time was considered as the time between the loss and the recovery of righting reflex<sup>7</sup>.

#### Effect on motor coordination measured by the Rota-rod test

This test was carried out with a rotating-rod Letica (4 cm diameter, 16 rev/min). Animals remaining on the rod for 3 min or more were selected and placed in groups of 4 mice each. The time of permanence on the rod was recorded 30 and 60 min after the administration of the extract. The number of failures was also recorded<sup>8</sup>.

### Statistical analysis

Differences with respect to control were evaluated using the Mann-Whitney U-test or Chi-square test (as applicable)<sup>9</sup>. Discrepances with  $p < 0.05$  were considered statistically significant.

### RESULTS AND DISCUSSION

-The administration of the algal extract produced a dose-dependent decrease in the number of head-dips. The maximum effect was observed 60 min after the administration of 25 mg/kg, the reduction being about 60% (Fig. 2).

All treatment with extract produced a significant dosage-dependent hypothermia. At 6.25 and 12.5 mg/kg the greatest drop occurred 30 min after administration, whereas at 25 mg/kg the effect was progressively observed up to 2 hours after injection, the maximum drop being about 5 °C (Fig. 3).

The extract only possessed a slight hypnotic effect at dose of 25 mg/kg, increasing the sleeping time about 60% (Table 1). No synergistic effect was observed when doses of 6.25 and 12.5 mg/kg were administered.

The administration of *Cystoseira usneoides* extract produced a dose-dependent effect in the time of stay on rod. At 6.25 mg/kg no significant activity was observed. At 12.5 mg/kg the reduction in the time on rod was significant 30 min after administration, whereas the number of animals unable to stay on the rod was significant 30 and 60 min after injection. At 25 mg/kg both time on rod and failures were significantly different from control 30 and 60 min after its administration (Table 2).

In conclusion, the extract obtained from *Cystoseira usneoides* decreased spontaneous locomotor and exploratory activities, indicating initially a central depressant effect accompanied with a slight hypnotic effect. The seaweed possessed a clear hypothermal activity and a very significant reduction in motor coordination which must be added too. Taken together the results obtained in these tests are indicative of a depressive action on CNS, invoking similar patterns of response to those characteristics of many groups of natural and synthetic depressants. Further investigations are necessary before any definitive conclusions can be drawn in this respect.

Fig 1.

Effect of *Cystoseira usneoides* extract on spontaneous locomotor activity. Each point represents the mean  $\pm$ S.E.M. of 5-6 groups of animals (4 mice/group). Significance of differences with respect to the control group was evaluated by the Mann-Whitney U-test: \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

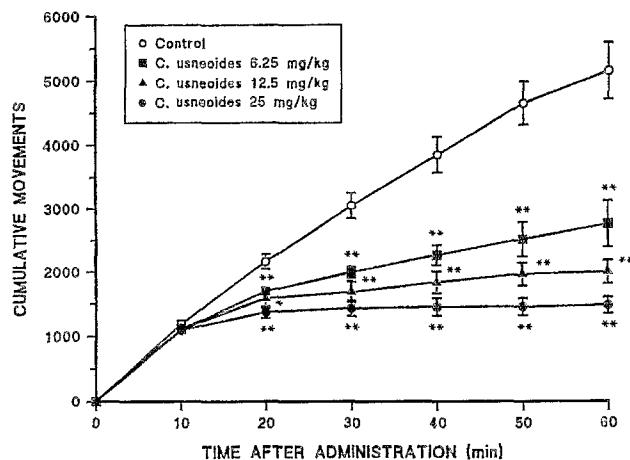
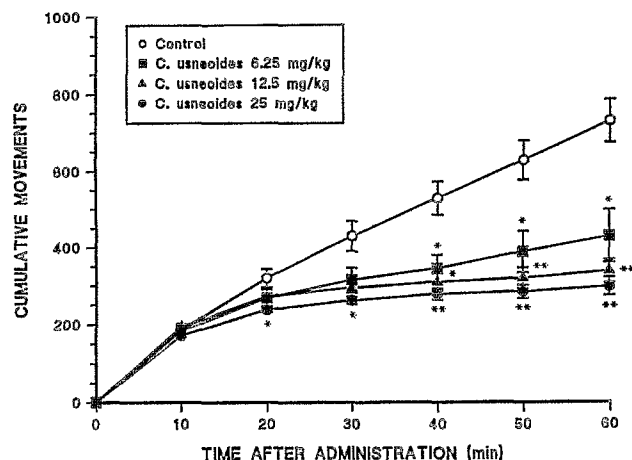


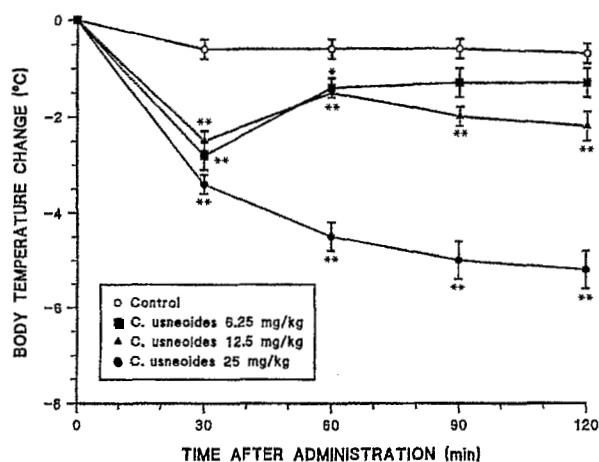
Fig 2.

Effect of *Cystoseira usneoides* extract on exploratory behaviour. Each point represents the mean  $\pm$ S.E.M. of 4-6 groups of animals (4 mice/group). Significance of differences with respect to the control group was evaluated by the Mann-Whitney U-test: \*  $p < 0.05$ ; \*\*  $p < 0.01$ .



**Fig 3.**

Effect of *Cystoseira usneoides* extract on rectal temperature. Each point represents the mean  $\pm$ S.E.M. of 7-8 animals. Significance of differences with respect to the control group was evaluated by the Mann-Whitney U-test: \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

**Table 1.**

Effect of *Cystoseira usneoides* extract on motor coordination. Each value represents the mean  $\pm$ S.E.M. of 4 groups of animals (4 mice/group). Significance of differences with respect to the control group was evaluated by the Mann-Whitney U-test (Stay on rod) and Chi-square test (Failures): \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

Treatment	Dosage (mg/kg)	+ 30 min		+ 60 min	
		Stay on rod (s)	Failures	Stay on rod (s)	Failures
Control	-	180.0 $\pm$ 0.0	0.0 $\pm$ 0.0	180.0 $\pm$ 0.0	0.0 $\pm$ 0.0
<i>C. usneoides</i> extract	6.25	155.4 $\pm$ 24.6	0.8 $\pm$ 0.8	161.8 $\pm$ 15.0	0.8 $\pm$ 0.5
	12.5	108.6 $\pm$ 13.3*	2.3 $\pm$ 0.5**	142.0 $\pm$ 16.3	1.5 $\pm$ 0.6*
	25	70.5 $\pm$ 17.2**	3.2 $\pm$ 0.5**	52.0 $\pm$ 14.7**	3.4 $\pm$ 0.2**

**Table 2.**

Effect of *Cystoseira usneoides* extract on pentobarbital-induced hypnosis. Each value represents the mean  $\pm$ S.E.M. of 7-8 animals. Significance of differences with respect to the control group was evaluated by the Mann-Whitney U-test: \*  $p < 0.05$ .

Treatment	Dosage (mg/kg)	Sleeping time (min)
Control	-	60.3 $\pm$ 7.1
<i>C. usneoides</i> extract	6.25	50.5 $\pm$ 4.1
	12.5	57.6 $\pm$ 13.4
	25	96.4 $\pm$ 16.2*

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