Determination of the Cytotoxicity of *Rumex crispus* during the Vegetation Period Using a Brine Shrimp Bioassay

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The percentage of anthraquinone glycosides in the roots of *Rumex crispus* L. (Polygonaceae) is significantly greater than in other parts of the plant. The roots of *R. crispus* in different vegetation stages were investigated with regard to anthraquinone glycoside content. Their toxicity towards shrimp were also tested during the vegetation period. The data showed that the percentage of anthraquinone glycosides as well as the toxic effect on the shrimp increased during the vegetation period.

Introduction

The roots of *Rumex* L. have been in traditional use, owing to their laxative properties (Van Os, 1976). We have previously shown that these roots have a significant anthraquinone glycoside content (Demirezer, 1994). As anthraquinones are known to be cytotoxic, with Emodin (1,6,8-trihydroxy-3-methyl anthraquinone) having an L_{50} value of 35 ppm (McLaughlin *et al.,* 1991), we have examined the toxic potential of *Rumex crispus* roots during the vegetation period, using the brine shrimp bioassay.

Materials and Methods

Plant materials

Rumex crispus samples were collected growing wild in empty lots in Ankara/Turkey from 15 May–30 August 1993.

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Test for cytotoxic activity (according to Meyer et al., 1982)

Extraction

Accurately weighed samples (200 mg) were heated with ethanol (20 ml) for 15 min under reflux. The extract was brought to room temperature filtered through Schleicher & Schüll 589[3] blue ribbon paper (110 mm ∅, Ref. No. 300210, Dassel, Germany) and taken to dryness *in vacuo.*

Sample preparation

The dried extract (20 mg) was dissolved in 2 ml ethanol (solution A). Solution B was prepared by 1/10 dilution of solution A and solution C was prepared by 1/10 dilution of solution B and solution D was prepared by 1/10 dilution of solution C, using ethanol. 0.5, 5, 50 and 500 µl aliquots of each solution were used to prepare 1, 10, 100 and 1000 ppm brine solutions (5 ml).

Hatching the shrimp

Brine shrimp eggs (*Artemia* sp.) (San Francisco Bay Brand Inc. Newark, CA 94560 U.S.A.) were hatched in a shallow rectangular dish filled with artificial sea water which was prepared with a commercial salt mixture (Artemia Salz: Hobby, Aus dem Hause Dohse Aquaristik, Bonn) and double-distilled water. After 48 h the phototropic nauplii which were separated from their shells in the divided tank, were collected from the illuminated side, using a capillary.

Bioassay

Ten shrimps were transferred to each sample vial in a capillary and artificial sea water was added to make 5 ml. The nauplii which were in the body of the capillary were counted after 48 h of illumination with an overhead fluorescent lamp (36 W) at a distance of 40 cm. The extract was added and the incubation was continued. After 48 h, percent deaths in controls and each dose of extract were determined.

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LC_{50} determinations

The data were analyzed with the FINNEY (probit analysis method) computer program (DOS) to determine LC_{50} values and 95% confidence intervals. (The Finney computer program was obtained from Prof. McLaughlin, Purdue University, West Lafayette, IN 47907 U.S.A.)

Results and Discussion

The anthraquinone glycoside content of the roots of *Rumex crispus* at different stages of vegetation was determined during a 4 month period extending from mid-May to the end of August, 1993. The roots were found to be rich in anthraquinones, the amount increasing significantly in August (Table I). The toxic potential of *Rumex* extracts, in terms of LC_{50} values relating to the brine shrimp assay are given in Table I. Comparison of anthraquinone content with toxic potential shows positive correlation. Different *Rumex* spec. are also under investigation.

The brine shrimp bioassay is not specific for antitumor or any particular physiological action; but it may be possible to screen biological activity using principally the brine shrimp bioassay, rather than other more expensive and time-consuming cytotoxic assays. A positive correlation has been found between toxicity towards brine shrimp and 9 KB (human nasopharyngeal carcinoma) cells which has suggests that this bioassay, functioned fairly well in the *in vitro* screening of cytotoxicity in fractionated plant extracts (McLaughlin, 1991).

Table I. Amount of anthraquinone glycoside in *Rumex crispus* root during the vegetation period and LC_{50} values.

Months	% of anthraquinone	Percent deaths after 24 h 1 [μg/ml]	10 [μg/ml]	100 [μg/ml]	1000 [μg/ml]	LC_{50}*	95% confidence interval
May	0.35	23.33	50	36.60	46.60	>1000	–
June	0.40	13.33	23.30	40	90	72.54	(32.91–177.16)
July	0.33	33.30	43.30	80	100	7.00	(2.60–15.12)
August	0.91	43.33	100	100	100	1.00	(0.30–1.90)

* μg dried extract/ml brine medium.

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