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EVALUATION OF THE PURIFIED FRACTION OF *WILBRANDIA (C.F.) VERTICILLATA* FOR ANTITUMOUR ACTIVITY

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Cucurbitacins are known to produce cytotoxic and anticancer activities. Two novel norcucurbitacin glucosides (Wv1 and Wv2) have recently been isolated from a purified fraction obtained from the rhizome of Wilbrandia verticillata. *The present study evaluates the cytotoxic and antitumour activities of these norcucurbitacins. We have found a regular cytotoxicity in KB cells (Cy_{50} = 12 μg/ml) as well as a significant inhibition in the Walker 256 carcinosarcoma growth (approximately 75%).*

Key words: antitumor – *Wilbrandia verticillata* – cytotoxicity – *Walker carcinosarcoma*

It is well known that plants can be a good source of antitumour drugs (Wall & Wani, 1977). The development of some new antineoplastic drugs has been obtained by chemical modification of already known active compounds or by the discovery of new molecular structures related to antitumous properties derived from plants (Arcamone & Cassinelli, 1980). The present work had its origins in an extensive research program involving Chemistry and Pharmacology of the Northeast plants which has been carried out since 1976. In connection with this program, a screening for cytotoxicity and anticancer activity has tested nearly 200 hydroalcoholic extracts (Moraes, 1981). The initial results indicated several plants with anticancer activity.

We describe in this article, the cytotoxicity and antitumour tests carried out on *Wilbrandia cf. verticillata* rhizome, a plant which belongs to the *Cucurbitaceae* family and is popularly known as "cabeça de negro". This plant is used as an antirheumatic remedy in Brazilian folk medicine. We have recently described (Matos et al., 1988) the preparation of a purified fraction from *Wilbrandia* rhizomes which, on the basis of spectroscopic data, seems to contain two novel compounds (Wv1 and Wv2) which are similar to the norcucurbitacins characterized by Achenbach et al. (1987).

MATERIALS AND METHODS

The tests for antitumour activity of *Wilbrandia verticillata* were carried out in Walker 256 carcinosarcoma model according to the "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems" (Geran et al., 1972). Tumour cell suspensions with 10^6 viable cells in 0.5 ml were injected intramuscularly into the inguinal region of the right thigh of the rats (6 to 8 weeks old). Before starting the treatment, an acute toxicity test was performed in mice. Afterwards, a daily dose of 50 mg per kg of body weight was administrated by intraperitoneal injection into the animals during 10 days. Cyclophosphamide in a dose of 2.5 mg per kg of body weight was used as positive control. The animals were sacrificed on the 11th day of the experiment and the tumour weight was obtained by the difference between the weight of the injected leg and the weight of a normal counterpart. The percentage of inhibition was calculated based on the following formula of Tarnowiski & Stock (1957), where *Tt* is the average of the tumour weight of the treated animals and *Tc* is the average of the tumour weight of the control animals.

$$\text{Effect} = \left(1 - \frac{Tt}{Tc}\right) . 100$$

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To detect possible subacute toxicity the weight of the liver and spleen, as well as the body weight were determined at the end of the experiment.

KB cells (Eagle, 1955), a human carcinoma cell line that has being maintained in our laboratory, were used for testing. The initial cell line of KB cells was obtained from the National Cancer Institute. The testing procedure was described previously (6). 0.1 ml of serial dilutions (100, 10, 1 e 0.1 μg/ml) was added to an sterile multiwell plate (24 rounded wells, 2 cm^2 of surface area and 1 ml of volume) of KB cells immediately after seeding 2 x 10^4/ml, and incubated for 4 days. The degree of cytotoxic effect (0 to 4 +) was determined by the morphological criteria, density of cells and degree of acid production (pH changes of medium) in comparison with the control cultures. Culture medium: Eagle's MEM supplemented with 3% fetal calf serum and gentamycin.

Statistical Analysis: experimental results were analyzed for their significance ($p < 0.05$) by Student's "t" test (2-tailed).

RESULTS AND DISCUSSION

The results of these preliminary experiments indicate that these two new norcucurbitacins possess significant antitumour activity and cytotoxicity. The antitumour test is shown on the Table.

TABLE

Tumour, spleen and liver weight of animals treated with *Wilbrandia verticillata* (10 mg/kg), cyclophosphamide and Ringer solution (control)

Group	Tumour (g)	Spleen (g)	Liver (g)
Ringer	57.0 ± 7.18	0.503 ± 0.62	44.5 ± 5.56
W. verticillata	20.2 ± 6.26	0.371 ± 0.61	36.8 ± 6.14
Ciclofosfamide	9.79 ± 2.34	0.328 ± 0.74	32.8 ± 6.05

The calculation of the inhibition showed 74.6% to *Wilbrandia verticillata* and 82.9% to cyclophosphamide.

Symptoms such as tremors, diarrhoea and weight loss were observed towards the end of the treatment in some animals.

A satisfactory cytotoxicity for *Wilbrandia verticillata*, which showed a Cy_{50} of 12 μg/ml, was found.

Several informations have been reported on the mechanism of action of the cucurbitacins, especially those related to alterations of the metabolic systems. It has been shown that cucurbitacins inhibit oxygen uptake in Ehrlich carcinoma cells (Shohat et al., 1967), and DNA, RNA and protein synthesis in Hela cells (Witkowski et al., 1984).

Although these are preliminary results, we can speculate that the antitumour activity of these two norcucurbitacin (Wv1 and Wv2) could be related to alterations of the metabolic system of the tumour cells.

Further studies should aim to isolate these novel cucurbitacins substances from the fraction and explore possibilities of improving their antitumour activity through chemical structural modifications.

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