Short communication

Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer

V. Steenkamp *, M.C. Gouws

Department of Urology, Faculty of Health Sciences, University of Pretoria, PO Box 667, Pretoria 0001, South Africa

Received 25 October 2005; accepted 23 February 2006

Abstract

Aqueous extracts prepared from six South African medicinal plants, with cancer-related ethnobotanical uses, were tested for their cytotoxic ability in vitro against three human cancer cell lines: DU-145 prostate cancer cells, MDA-MB-231 and MCF-7 breast cancer cells and a non-malignant breast cell line, MCF-12A. The plants studied were: Bidens pilosa, Centella asiatica, Cnicus benedictus, Dicoma capensis, Hypoxis hemerocallidea and Sutherlandia frutescens. Of these plants, only D. capensis exhibited pronounced cytotoxic effects in two of the cell lines tested: MCF-7 and MCF-12A.

© 2006 SAAB. Published by Elsevier B.V. All rights reserved.

Keywords: Cancer; Cytotoxicity; Medicinal plants; South Africa

1. Introduction

Reports on plants used for the treatment of cancer are rare in South Africa. Mongelli et al. (2000) found this to be true for Argentina plants, and ascribed it to the fact that cancer involves a complex set of signs and symptoms. It has been recommended that ethnopharmacological usages such as immune and skin disorders, inflammatory, infectious, parasitic and viral diseases be taken into account when selecting plants used to treat cancer, since these reflect disease states bearing relevance to cancer or a cancer symptom (Cordell et al., 1991; Popoca et al., 1998).

Since the majority of cancer chemotherapeutants severely affect the hosts’ normal cells (Mascarenhas, 1994), the use of natural products has now been contemplated of exceptional value in the control of cancer (Suffness and Pezzuto, 1990). Furthermore, the search for new sources of biologically active compounds is important for the discovery of new drugs for the treatment of cancer.

This study determined the cytotoxic activities in aqueous extracts of six plants used by traditional healers in South Africa to treat cancer. The plants investigated were: (i) leaves and stems of Bidens pilosa L. (Asteraceae, Mai-Mai market, Johannesburg) used in the treatment of prostate gland tumours and inflammation (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996); (ii) Centella asiatica (L.) Urb. (Araliaceae, South African National Biodiversity Institute (SANBI), Tshwane) leaves which are prescribed for skin complaints, rheumatoid arthritis, cancer and fevers (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996); (iii) decoctions of Cnicus benedictus L. (Asteraceae, SANBI, Tshwane) taken for the treatment of internal cancer (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997); (iv) leaves and twigs of Dicoma capensis Less. (Asteraceae, gift from Prof. B-E van Wyk, Department of Botany, Rand Afrikaans University) which are prescribed in cases of cancer, high blood pressure and fever (Van Wyk et al., 1997), (v) Hypoxis hemerocallidea Fisch. and C.A. Mey. (Hyloxidaceae, Mai-Mai market, Johannesburg) corms used to treat bladder disorders and testicular tumours (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997) and (vi) leaf decoctions of Sutherlandia frutescens (L.) R.Br. (Fabaceae, provided by Dr. C. Albrecht, Medical Research Council) which are taken for stomach problems, internal cancer, inflammation and viral diseases (Watt and Breyer-Brandwijk, 1962; Rood, 1994; Van Wyk et al., 1997). Cytotoxicity was determined against a prostate carcinoma cell line (DU-145), breast cancer cell lines (MCF-7 and MDA-MB-231) and a non-malignant breast cell line (MCF-12A).
All the remedies investigated in this study are prescribed as infusions prepared by traditional healers, therefore, only aqueous extracts were tested in vitro. Dried plant material (1 g) was suspended in 10 ml deionised water and brewed as a tea by boiling for 15 min. Extracts were allowed to cool, centrifuged and the supernatants passed through 0.45 μm and 0.22 μm filters, consecutively. The yields were 0.75%, 2.85%, 2.49%, 0.55%, 3.69% and 2.38% for B. pilosa, C. asiatica, C. benedictus, D. capensis, H. hemerocallidea and S. frutescens, respectively.

Human DU-145 prostate carcinoma cells were maintained in Ham’s F10 nutrient mixture supplemented with 5% decomplemented foetal calf serum (FCS), gentamycin sulphate (0.004%), glucose (0.57%) and NaHCO₃ (0.12%). MCF-12A cells were cultured in a 1:1 mixture of Dulbecco’s minimum essential medium (DMEM) and Ham’s F12 medium supplemented with epidermal growth factor (20 ng/ml), cholera toxin (100 ng/ml), hydrocortisone (500 ng/ml) and FCS (10%). MDA-MB-231 and MCF-7 breast carcinoma cells were grown in DMEM supplemented with sodium pyruvate (111 mg/l), sodium bicarbonate (2.25 g/l) and 10% FCS.

Cells were seeded into 96-well flat-bottomed plates at a concentration of 3.0 × 10⁵ cells per ml. After 24 h, cells were treated with plant extract, which was diluted with culture medium to a final concentration of 50 μg/ml (Zee-Cheng, 1997; Itharat et al., 2004). XTT labelling reagent (50 μl) was added and the absorbance (560 nm) read after 72 h (Gerlier and Thomasset, 1986). Experiments were carried out three times in triplicate. Active extracts were considered as those with less than 50% survival after an exposure time of 72 h. Cisplatin, a known anti-tumour agent, was used as positive control.

The effects of the plant extracts on proliferation in DU-145, MDA-MB-231, MCF-7 and MCF-12A cells are presented in Fig. 1A–D, respectively. Results showed B. pilosa to inhibit proliferation by 10% in the carcinoma cell lines. Inhibition of proliferation of leukemia cells in vitro has been reported with hot water extracts of this plant (Chang et al., 2001). In four of the five leukemia cell lines tested by these authors, the IC₅₀ ranged between 145 and 200 μg/ml, concentrations 3–4 fold above that tested in the present study. There are reports that describe B. pilosa as having antiviral (Chiang et al., 2003), antileukemic (Chang et al., 2001), anti-bacterial (Rabe and van Staden, 1997), anti-malarial (Brandao et al., 1997), antioxidative (Chiang et al., 2004), immunosuppressive and anti-inflammatory activity (Jäger et al., 1996; Pereira et al., 1999). The extracts of H. hemerocallidea stimulated DU-145 and MCF-12A cell growth and inhibited the growth of the MCF-7 cells. This plant has been reported to display anti-inflammatory activity (Ojewole, 2002), an activity related to cancer. The aqueous extracts of S. frutescens inhibited growth of the oestrogen dependent cancer cell lines and stimulated the growth of the MCF-12A and MDA-MB-231 cells. Chinkwo (2005) found the crude aqueous whole plant extract of S. frutescens to induce cytotoxicity in cervical carcinoma and Chinese Hamster Ovary cells. Ethanolic extracts of S. frutescens commercial preparations (tablets and powder) have been reported to inhibit proliferation of both MCF-7 and MDA-MB-468 human breast cancer cells.

Fig. 1. The effect of 50 μg/ml plant extract on (A) DU-145 prostate cancer cells, (B) MDA-MB-231 breast cancer cells, (C) MCF-7 breast cancer cells and (D) MCF-12A non-malignant breast cells after an exposure time of 72 h; (A) Bidens pilosa; (B) Hypoxis hemerocallidea; (C) Sutherlandia frutescens; (D) Centella asiatica; (E) Cnicus benedictus; (F) Dicoma capensis. The values are expressed as means±S.E.M. (n=3).
cancer cells, human leukemia Jurkat cells, human promyelocyte HL60 cells and murine RAW 264.7 macrophage/monocyte cells (Tai et al., 2004). *S. frutescens* has been shown to possess anti-oxidant potential (Fernandes et al., 2004), which is thought to account for some of the anti-inflammatory properties already known (Ojewole, 2004). In this study extracts of *C. asiatica* stimulated the growth of three of the cell lines tested. Crude and partially purified fractions of a methanolic extract of *C. asiatica* have been reported to inhibit proliferation of transformed cell lines (Ehrlich ascites tumour cells: IC₅₀ 62 μg/ml, Dalton’s lymphoma ascites tumour cells: IC₅₀ 75 μg/ml), and to be non-toxic to normal human lymphocytes (Babu et al., 1995). Antifungal, antibacterial, anti-inflammatory and anti-allergic activity has been described for *C. asiatica* (Ponglux et al., 1987). *C. benedictus* and *D. capensis* inhibited the proliferation of DU-145, MCF-7 and MCF-12A cells. We found no literature where the cytotoxic activity of *C. benedictus* or *D. capensis* has previously been investigated.

The American National Cancer Institute guidelines set the limit of activity for crude extracts at a 50% inhibition (IC₅₀) of proliferation of less than 30 μg/ml after an exposure time of 72 h (Suffness and Pezzuto, 1990). With the exception of *D. capensis*, the other plant species had IC₅₀ values higher than 50 μg/ml. The IC₅₀ values of the latter plant were 30 μg/ml and 31 μg/ml in MCF-7 and MCF-12A cells, respectively. The positive control, cisplatin, had IC₅₀ values of 0.27 μg/ml and 0.14 μg/ml in MCF-7 and MCF-12A cells, respectively.

Some of the remedies investigated in this study have been studied from a chemical point of view. The active substances of *H. hemerocallidea* (rooperol), *C. benedictus* (cinicin, arctigenin, arctinin) and *S. frutescens* (canavanine) are known to exhibit tumouricidal or cytotoxic activity (Nicoletti et al., 1992; Hirano et al., 1994; Southon, 1994; Swaffar et al., 1994; Moritani et al., 1996). *B. pilosula* contains phenylethylamine which amongst others kills human fibroblast cells (Morton, 1962). Asiaticoside isolated from *C. asiatica* is reported to possess an IC₅₀ of 1.58 ± 0.15 mg/ml in MCF-7 cells (Huang et al., 2004).

Since cytotoxicity of the plants investigated has been reported by other authors, it is evident that different cell lines exhibit different sensitivities towards the plant extracts. Also, some plants are reported to have a cytotoxic effect on cancer cells (Kusuge et al., 1985; Alley et al., 1988) whereas other plant extracts activate several parameters of the immune system as a strategy to destroy cancer (Abuharfeil et al., 2000). Differences in results obtained in this study and those reported in the literature could also be ascribed to differences in extraction procedures and the natural variability in plants.

Since *D. capensis* showed the most pronounced cytotoxic activity, this plant will be evaluated further for the possible isolation of active compounds.

**Acknowledgements**

The Cancer Association of South Africa, Struwig-Germshuysen and the Wolmarans Research Trust are thanked for financial support.

**References**

Abuharfeil, N.M., Maraqa, A., Von Kleist, S., 2000. Augmentation of natural killer cell activity in vitro against tumor cells by wild plants from Jordan. Journal of Ethnopharmacology 71, 55–63.

Alley, M.C., Scudiero, D.A., Monks, A., Hursey, M.L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Shoemaker, R.H., Boyd, M.R., 1988. Feasibility of drug screening with panels of tumor cells using a microculture of tetracyclous assay. Cancer Research 48, 589–601.

Babu, T.D., Kuttan, G., Padikkala, J., 1995. Cytotoxic and anti-tumour properties of certain taxa of Umbelliferae with special reference to *Cen-tella asiatica* (L.) urban. Journal of Ethnopharmacology 48, 53–57.

Brandao, M.G.L., Kretti, A.U., Soares, L.S.R., Nery, C.G.C., Marinuzzi, H. C., 1997. Antimalarial activity of extracts and fractions from *Bidens pilosa* and other *Bidens* species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds. Journal of Ethnopharmacology 57, 131–138.

Chang, J.S., Chang, L.C., Chen, C.C., Liu, L.T., Wang, K.C., Lin, C.C., 2001. Antileukemic activity of *Bidens pilosa* L. var. minor (Blume) Sherff and *Houtanyng cordata* Thunb. American Journal of Chinese Medicine 29, 303–312.

Chiang, L.C., Chang, J.S., Chen, C.C., Ng, L.T., Lin, C.C., 2003. Anti-herpes simplex virus activity of *Bidens pilosa* and *Houtanyng cordata*. American Journal of Chinese Medicine 31, 355–362.

Chiang, Y.M., Chuang, D.Y., Wang, S.Y., Kuo, Y.H., Tsai, P.W., Shyur, L.F., 2004. Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*. Journal of Ethnopharmacology 95, 409–419.

Chinkwo, K.A., 2005. *Sutherlandia frutescens* extracts can induce apoptosis in cultured carcinoma cells. Journal of Ethnopharmacology 98, 163–170.

Cordell, G.A., Beecher, C.W., Pezzut, J.M., 1991. Can ethnopharmacology contribute to development of new anti-cancer? Journal of Ethnopharmacology 32, 117–133.

Fernandes, A.C., Cromarty, A.D., Albrecht, C., Jansen van Rensburg, C.E., 2004. The antioxidant potential of *Sutherlandia frutescens*. Journal of Ethnopharmacology 95, 1–5.

Gerlier, D., Thomasset, N., 1986. Use of MTl colorimetric assay to measure cell activation. Journal of Immunological Methods 94, 57–63.

Hirano, T., Gotoh, M., Oka, K., 1994. Natural flavonoids and lignans are potent cytostatic agents against human leukemic cells HL-60. Life Sciences 55, 1061–1069.

Huang, Y.H., Zhang, S.H., Zhen, R.X., Xu, X.D., Zhen, Y.S., 2004. Asiaticoside inducing apoptosis of tumor cells and enhancing anti-tumor activity of vincristine. Ai Zheng 23, 1599–1604.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg.

Itharat, A., Houghton, P.J., Eno-Amoquay, E., Burke, P.J., Sampson, J.H., Raman, A., 2004. In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. Journal of Pharmacology 90, 33–38.

Jäger, A.K., Hutchings, A., van Staden, J., 1996. Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. Journal of Ethnopharmacology 52, 95–100.

Kusuge, T., Yokota, M., Sugiyama, K., Yamamoto, T., Yan, S.C., 1985. Studies on antitumor activities and antitumor principles of Chinese herbs. Yakogako Zasshi 105, 791–795.

Mascarenhas, M., 1994. Structure–activity characterization, a quick method to screen mushrooms for the presence of antimycobacterial glucans. Mushroom Research 3, 77–80.

Mongelli, E., Pampuro, S., Coussio, J., Salomon, H., Ciccia, G., 2000. Cytotoxic and DNA interaction activities of extracts from medicinal plants used in Argentina. Journal of Ethnopharmacology 71, 145–151.

Moritani, S., Nomura, M., Takeda, Y., Miyamoto, K., 1996. Cytotoxic components of *Bardanae fructus* (goboshi). Biological and Pharmaceutical Bulletin 19, 1515–1517.

Morton, J.F., 1962. Spanish needles (*Bidens pilosa*) as a wild food resort. Economic Botany 16, 173–179.

Nicoletti, M., Galeffi, C., Messana, I., Marini-Bettolo, G.B., 1992. Hypoxi-daceae. Medicinal uses and the norlignan constituents. Journal of Ethnopharmacology 36, 95–101.
Ojewole, J.A., 2002. Anti-inflammatory properties of *Hypoxis hemerocallidea* corm (African potato) extracts in rats. Methods and Findings in Experimental and Clinical Pharmacology 24, 685–687.

Ojewole, J.A., 2004. Analgesic, anti-inflammatory and hypoglycaemic effects of *Sutherlandia frutescens* R. Br. (variety Incana E. Mey.) [Fabaceae] shoot aqueous extract. Methods and Findings in Experimental and Clinical Pharmacology 26, 409–416.

Pereira, R.L., Ibrahim, A.T., Lucchetti, L., da Silva, A.J., Goncalves de Moraes, V.I., 1999. Immunosuppressive and anti-inflammatory effects of methanolic extract and the polyacetylene isolated from *Bidens pilosa* L. Immunopharmacology 43, 31–37.

Ponglux, D., Wong, S., Phadungcharoen, T., Ruangrungsri, N., Likhitwitayawud, K., 1987. Medicinal Plants. The First Princess Chulabhorn Science Congress Bangkok, Thailand.

Popoca, J., Aguilar, A., Alonso, D., Villarreal, M.L., 1998. Cytotoxic activity of selected plants used as antitumourals in Mexican traditional medicine. Journal of Ethnopharmacology 59, 173–177.

Rabe, T., van Staden, J., 1997. Antibacterial activity of South African plants used for medical purposes. Journal of Ethnopharmacology 56, 81–87.

Rood, B., 1994. Uit die Veldapteek. Tafelberg Publishers, Cape Town, South Africa.

Southon, I.W., 1994. Systematic significance of canavine in the Papilionoideae (Faboideae). Biochemical Systematics and Ecology 6, 201–212.

Suffness, M., Pezzuto, J.M., 1990. Assays related to cancer drug discovery. In: Hostettmann, K (Ed.), Methods in Plant Biochemistry: Assays for Bioactivity, vol. 6. Academic Press, London, pp. 71–133.

Swaffar, D.S., Ang, C.Y. Desai, P.B., Rosenthal, G.A., 1994. Inhibition of the growth of human pancreatic cancer cells by the arginine antimebolite l-canasavine. Cancer Research 54, 6045–6048.

Tai, J., Cheung, S., Chan, E., Hasman, D., 2004. In vitro culture studies of *Sutherlandia frutescens* on human tumor cell lines. Journal of Ethnopharmacology 93, 9–19.

van Wyk, B.-E., van Oudshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. Livingstone, London.

Zee-Cheng, R., 1997. Anticancer research on Loranthaceae plants. Drugs of the Future 22, 519–530.

Edited by P.J. Houghton