TOXICOLOGICAL SCIENCES **70,** 245–251 (2002)

The Popular Herbal Antimalarial, Extract of *Cryptolepis sanguinolenta*, Is Potently Cytotoxic

Charles Ansah and Nigel J. Gooderham[1]

Molecular Toxicology, Faculty of Medicine, Sir Alexander Fleming Building, Imperial College of Science, Technology and Medicine, Exhibition Road, London, SW7 2AZ, UK

Received August 1, 2002; accepted September 12, 2002

The aqueous root extract of *Cryptolepis sanguinolenta* (CSE) is a popular antimalarial in West African ethnomedicine. Cryptolepine (CLP), the major alkaloid of the plant, is a cytotoxic DNA intercalator that has promise as an anticancer agent. To date the aqueous root extract, the traditional antimalarial formulation, has not been evaluated for toxicity. In this study, we have examined the *in vitro* toxicity of CSE and CLP using V79 cells, a Chinese hamster lung fibroblast frequently used to assess genetic toxicity, and a number of organ-specific human cancer cell lines. CSE and CLP caused a dose- and time-dependent reduction in viability of the V79 cell line. Flow cytometric analysis of CSE- and CLP-treated (24 h) asynchronously growing V79 cells using propidium iodide (PI) staining revealed an accumulation of cells (up to 55%) in the sub-G1 phase of the cell cycle, indicative of cell death. The V79 cells and almost all the organ-specific human cancer cell lines exposed to CSE and CLP were profoundly growth inhibited, as measured in a clonogenicity assay. In a V79 cell mutation assay (*hprt* gene), CSE (5–50 μg/ml) only induced mutation at the highest dose employed (mutation frequency ~4 and 38 mutant clones per 10^6 cells for control and CSE, respectively), but CLP (0.5–5.0 μM) was not mutagenic. These results indicate that CSE and CLP are very cytotoxic and may be weak mammalian mutagens and/or clastogens. The poor genotoxicity of CSE and CLP coupled with their potent cytotoxic action support their anticancer potential.

***Key Words:* *Cryptolepis sanguinolenta*; cryptolepine; cytotoxicity; *hprt* mutation; alamar blue; Trypan blue; clonogenicity.**

The search for effective and safer anticancer remedies is at the forefront of scientific research, worldwide. Botanical products as a source have contributed immensely to the current stock of anticancer agents, and research efforts directed at discovering new agents from this source are important. *Cryptolepis sanguinolenta* (Periplocaceae) is an established antimalarial in West African ethnomedicine (Boye and Ampofo, 1983), but is also thought to be a promising anticancer agent. Used traditionally in the form of the aqueous extract of the roots, unconfirmed claims by traditional medical practitioners in Ghana suggest that it is effective in the management of breast tumors and related diseases. The major alkaloid of the roots, cryptolepine (CLP) (Dwuma-Badu *et al*., 1978; Tachie *et al*., 1991), is reported to possess a multiplicity of biological effects, reviewed in Bierer *et al*., (1998), that include antimicrobial, antimuscarinic, vasodilating, noradrenergic, antithrombotic, antiinflammatory, and hypoglycemic activities. CLP also exhibits cytotoxic and topoisomerase II inhibitory actions, intercalates and inhibits DNA synthesis (Bonjean *et al*., 1998) and induces apoptosis in HL-60 leukemia cells (Dassonneville *et al*., 2000). In a recent report, the CLP-DNA interaction was described as unique compared to other known modes of DNA intercalation (Lisgarten *et al*., 2002). In view of the traditional medicinal claims and previous reports on the toxicity of CLP, we have examined the *in vitro* toxicity of the aqueous extract of *Cryptolepis sanguinolenta* (the traditionally used clinical formulation, CSE) and CLP on V79 cells, a Chinese hamster lung fibroblast cell line.

MATERIALS AND METHODS

Preparation of the aqueous extract of CSE. To simulate the local method of preparation, roots of *Cryptolepis sanguinolenta* obtained from Mampong Akwapim, Ghana, were washed thoroughly with water, cut into small pieces, air dried for two weeks and powdered. A 10% w/v aqueous suspension of the powdered material was boiled for an hour, filtered, and the filtrate freeze-dried to produce CSE, the standardized aqueous preparation used in the current studies.

Chemicals. Synthetic CLP (purity >99%) was a kind donation from Dr. J. Addae-Kyeremeh, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. CSE and CLP were dissolved in cell culture media and filtered through sterile 0.2 μM filter for cell culture studies. Unless otherwise indicated in the text, all other chemicals were purchased from Sigma Chemical Co. (Poole, England.)

Cell line and conditions. The V79-MZ cell, a Chinese hamster lung fibroblast cell line was propagated in Dulbecco's modified Eagle's medium (DMEM); HCT116, a human colon adenocarcinoma cell line in RPMI; SKOV3, a human ovary adenocarcinoma cell line in Macoy's 5a; MCF7, a human breast adenocarcinoma in minimal essential medium (MEM), and MDA MB 361, a human breast adenocarcinoma cell line in L-15 media, all from Invitrogen Corporation (Paisley, Scotland, UK). The media were supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, and 100 units of penicillin/streptomycin, also from Invitrogen.

[1] To whom correspondence should be addressed. Fax: (44) 020 7594 3050. E-mail: n.goooderham@ic.ac.uk.

Routinely, cells were maintained at 37°C in a humidified atmosphere of 5% CO_2/95% air, harvested by trypsinization with trypsin-EDTA (Invitrogen) and centrifugation, and subcultured as appropriate for the individual cell lines. Cell numbers were determined by counting with a haemocytometer and viability was assessed using Trypan blue exclusion.

Growth inhibition (resazurin reduction assay). Growth inhibition was assessed by serial measurements of the fluorescence intensity following the reduction of resazurin (alamar blue) by viable cells (Fields and Lancaster, 1993). Briefly, V79 cells were seeded into 24-well multiwell dishes at a density of 5×10^4 cells per well in culture medium and then incubated overnight before treatment with vehicle, CSE, or CLP for 4, 24, or 72 h. After the incubation period, 1% v/v alamar blue was added to each well and incubated for a further 1 h. The fluorescence intensity was then measured using a plate reader, Fluostar® with excitation at 530 nm and emission at 590 nm. Under these conditions the cells remain viable and continue to grow, allowing serial fluorescent measurements to be recorded.

Cell viability (Trypan blue staining). In order to estimate the percentage of dead V79 cells after treatment with CSE or CLP, floating cells in the medium of each flask were transferred to centrifuge tubes. After detachment of the adherent cells with trypsin, the cells were mixed with the corresponding floating cells before centrifugation. The cells were then stained with 0.4% Trypan blue, and the number of Trypan blue positive and negative cells were counted on a haemocytometer by light microscopy.

Colony survival (clonogenic assay). For survival studies, treated cells were trypsinized, centrifuged, and reseeded at 100 cells per well in 6-well multidishes in drug-free medium. After incubation for at least 7 days, the wells were stained with methylene blue in 50% methanol, and colonies that contained 50 or more cells were scored as survivors. Cell survival was expressed as a percentage of appropriate vehicle-treated controls.

Flow cytometry analysis. Vehicle-, CSE-, or CLP-treated V79 cultures were harvested by centrifugation, washed twice with PBS, and fixed in 70% ethanol at –20°C for 24 hours. Cells were then resuspended in 1 ml of PBS solution containing 5 μg/ml propidium iodide (PI) and 100 μg/ml RNase and incubated in a water bath at 37°C for 30 minutes. Samples were then analyzed on a Becton Dickinson FACScan flow cytometer. Using the Cellquest software, the percentage of cells at different phases of the cell cycle was determined. PI was excited at 488 nm, and fluorescence analyzed at 620 nm.

Hprt mutation assay. The assay was performed according to the method of Yadollahi-Farsani *et al*. (1996) with modifications. Prior to drug treatment, V79-MZ cells were maintained in culture medium supplemented with HAT (5×10^{-5} M hypoxanthine, 4×10^{-7} M aminopterin, and 5×10^{-6} M thymidine) for 72 h to reduce the frequency of preexisting *hprt* mutants in the cell population. Cells in exponential phase (1.5×10^6) were seeded into 75-cm^2 flasks in culture medium 24 h before treatment with CSE, CLP, or the positive control, ethylmethane sulphonate (EMS) for a further 24 h.

To determine cell survival, treated cell cultures were trypsinized and reseeded at 100 cells/well in 2 ml of culture medium in 6-well plates. At the end of 7 days, wells were stained with methylene blue in 50% methanol and colonies that contained more than 50 cells were scored as survivors. Cell survival was expressed as a percentage of vehicle-treated controls.

Resistance to the lethal effects of the purine analogue 6-thioguanine (6-TG) was used as the genetic marker for the measurement of mutant frequency (MF). The treated cells were maintained in exponential growth for 7 days to allow phenotypic expression of induced mutants. Following the expression period, 1×10^6 cells were plated at 2×10^3 cells/cm^2 in culture media containing 5 μg/ml of 6-TG for a period of 7–10 days. The cloning efficiency at the time of mutant selection was also determined in normal culture medium (41 ± 4%) to correct for the observed mutant frequencies.

Statistical analysis. Samples were compared using the Student's *t*-test.

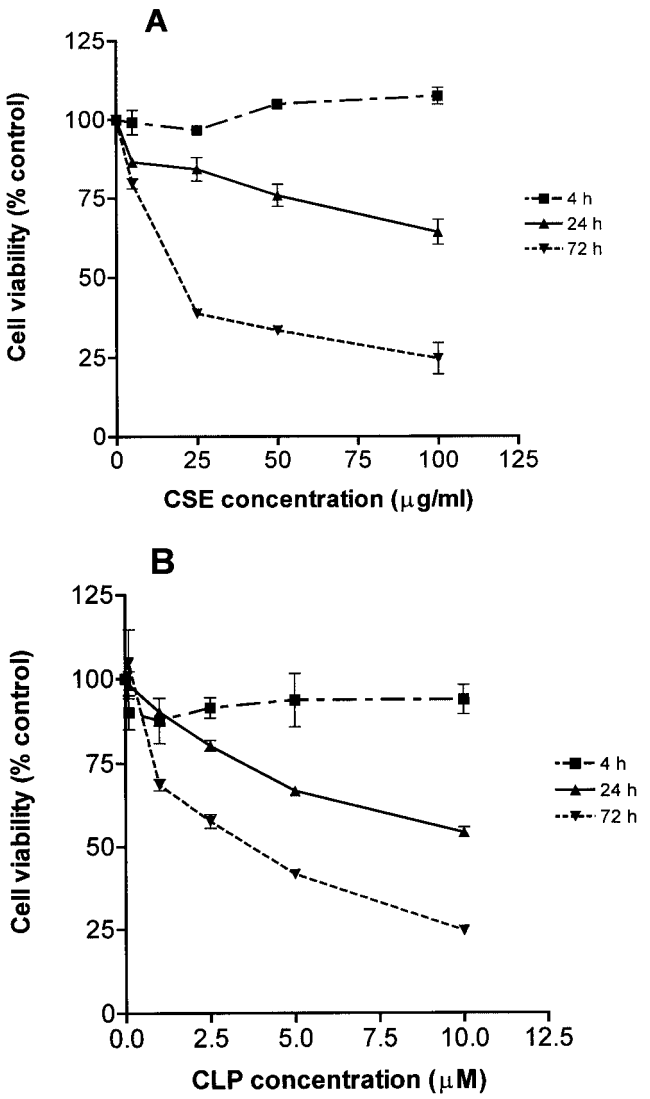

FIG. 1. Growth inhibition of V79 cells treated with CSE (A) and CLP (B) using the resazurin (alamar blue) method. Cells were exposed continuously for 4, 24, or 72 h as described in Materials and Methods. Values are means of 3 separate experiments, each in quadruplicate, and bars are standard deviations. Controls were treated with vehicle alone.

RESULTS

Growth inhibition. In the resazurin reduction assay, viable cells reduce the nonfluorescent dye resazurin (alamar blue) to resorufin, a highly fluorescent compound. The fluorescence measured depends on the viability of the cells (Fields and Lancaster, 1993). The relative fluorescence (RF) was determined as the sample fluorescence expressed as a percentage of vehicle-treated control. Continuous treatment of V79 cells for 24 or 72 hours with CSE or CLP caused a dose- and time-dependent growth inhibition of the cell line. Four-hour treatment was without effect over the concentration range used (Figs. 1A and 1B). The IC_{50} values for CSE and CLP were

estimated as 32.9 μg/ml and 4.3 μM respectively for V79 cells treated for 72 hours. The pattern of growth inhibition was remarkably similar for CSE- and CLP-treated cells.

Cell viability by Trypan blue exclusion. It was not clear from the alamar blue study if the observed growth inhibition was due to growth arrest or cell death since both could result in reduced cell numbers and an apparent loss of viability. To directly assess viability, a Trypan blue exclusion test was performed. The number of dead cells was observed to be dose- and time-dependent. After 24 hours of incubation with CSE or CLP, the percentage of dead cells was up to 30% at the highest dose of CSE or CLP tested (100 μg/ml and 5 μM, respectively) (Figs. 2A and 2B). This correlated well with the alamar blue data (Figs. 1A and 1B). The percentage of dead cells, however, increased dramatically to almost 100% after a 72-hour incubation, reflecting the time-dependent loss of viability.

Colony survival (clonogenic assay). To assess the long-term survival of the cells after treatment, we performed clonogenic assays on V79 cells and selected organ-specific human cancer cell lines. Both CSE and CLP inhibited the colony-forming ability of V79 cells in a dose- and time-dependent manner (Figs. 3A and 3B). Cells treated for 4 h, which appeared unaffected in the alamar blue test (Figs. 1A and 1B), showed a reduced long-term survival, suggesting that the treatment induced insidious cell damage. After 24 hours of incubation, none of the V79 cells treated with 100 μg/ml CSE or 5 μM CLP were able to generate colonies in sharp contrast to the seemingly 70% viability in the Trypan blue test. The IC_{50} values for V79 cells and the selected organ-specific human cancer cell lines (Table 1) show variability in the sensitivity of the different cell lines to CSE and CLP when compared to V79 cells.

Assessment of cell cycle distribution by flow cytometry. To determine whether treatment with CSE or CLP could affect the progression through the cell cycle of asynchronously growing V79 cells, cells treated for 24 h and vehicle-treated cells were stained with propidium iodide (PI) as described in Materials and Methods and the cell cycle status determined by flow cytometry. The DNA histograms obtained (Fig. 4) indicate that treatment with 5 μg/ml CSE or 0.5 μM CLP for 24 hours did not appear to have any significant effect on the cell cycle distribution. However, beyond these doses a gradual accumulation of sub-G1 cell population began to emerge in a dose-dependent fashion, accounting for about 55% of the population after treatment with 50 μg/ml CSE or 5 μM CLP. These sub-G1 cells are most likely apoptotic (Darzynkiewicz *et al.*, 1992). This observation was in sharp contrast to our results for the Trypan blue test, where we observed only 30% cell death. This suggests that a significant number of the dying cells excluded Trypan blue and were probably early apoptotic. Confirmation of apoptosis was obtained by assessing morphological change using light microscopy and Diff-Quick® (Dade Behring, Switzerland) (data not shown). Again, the effects of CSE and CLP on the cycle distribution were strikingly similar.

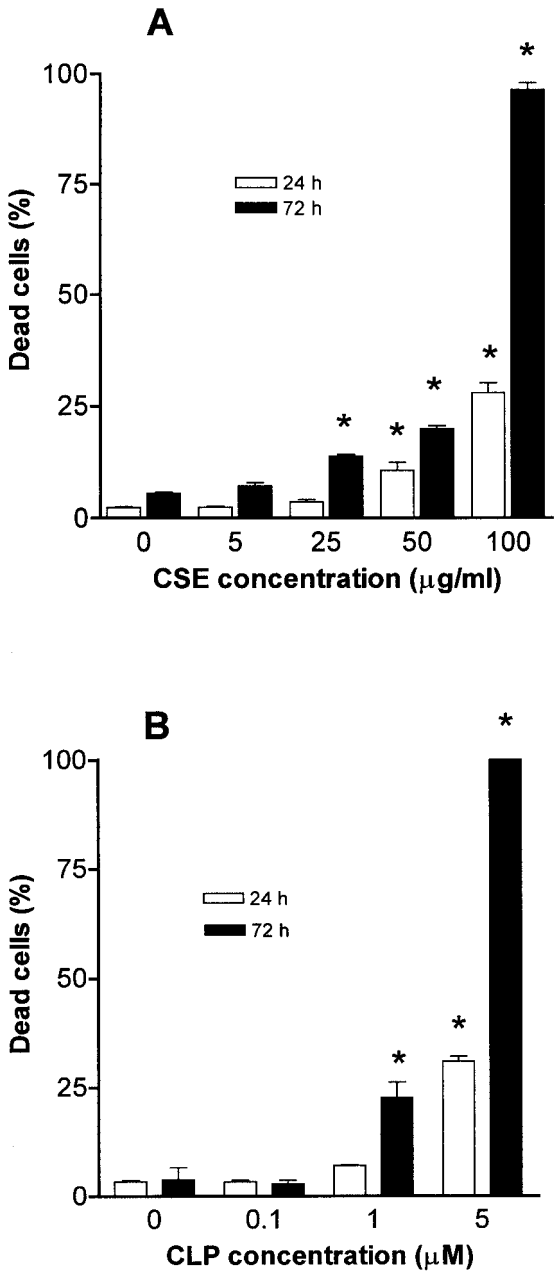

FIG. 2. Cell viability of V79 cells treated with CSE (A) and CLP (B) as determined by the Trypan blue exclusion assay. Cells were treated for 24 or 72 h, and then floating cells were mixed with adherent cells before centrifugation. Cells were counted manually under a light microscope after staining with 0.4% Trypan blue. Values are the mean of 3 separate experiments, each in duplicate, and bars are standard deviations. *Significant compared to control, $p < 0.01$.

Hprt mutation. To determine if the reported interaction of CLP and DNA (Bonjean et al., 1998; Lisgarten et al., 2002) could lead to genotoxicity, we used the *hprt* mutation assay (Yadollahi-Farsani et al., 1996), which is capable of detecting

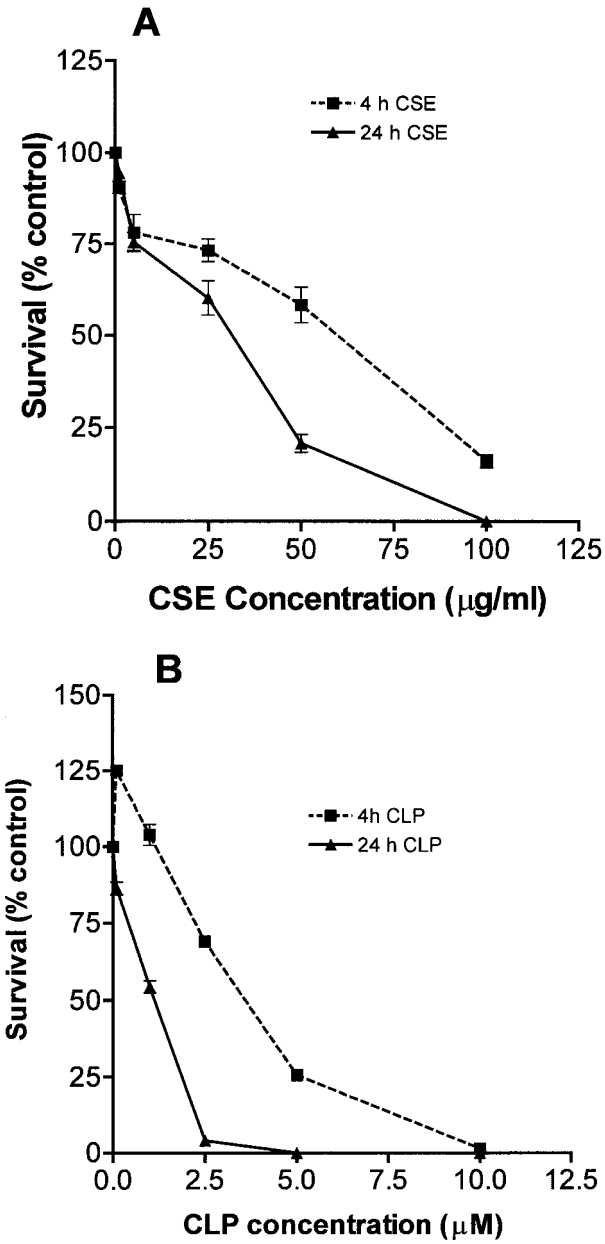

FIG. 3. Clonogenicity of V79 cells after 4- and 24-h treatment with CSE (A) and CLP (B). Cells were washed and plated at 100 cells/well in 6-well multidishes immediately after treatment in drug-free medium; colonies were scored after 7 days as described. The points represent the mean values obtained from 3 separate experiments, each in triplicate. Bars are standard deviations.

the mutagenicity induced by different DNA-damaging agents (Thacker and Ganesh, 1989). In this assay, resistance to the lethal effects of the purine analogue 6-thioguanine is used as the genetic marker for the selection of *hprt* mutants. As expected, the positive control EMS produced a high MF in the order of 163×10^{-6} surviving cells. CLP did not appear to be mutagenic in this assay; however, a significant MF of 38×10^{-6} was obtained at the highest dose of CSE (50 μg/ml) after correction for cell survival (Figs. 5A and 5B). Under the conditions of the assay, cloning efficiency was 41 $\pm$ 4%.

DISCUSSION

The aqueous extract of *Cryptolepis sanguinolenta* is an established antimalarial in the West African subregion (Boye and Ampofo, 1993), and there are several reports on the antimalarial activity of its major alkaloid CLP (Kirby *et al*., 1995; Noamesi *et al*., 1991; Wright *et al*., 1996). In the present study, we have used several different measures of cytotoxicity to demonstrate that the traditional clinically used antimalarial formulation, CSE, is profoundly cytotoxic to mammalian cells. We have also confirmed the cytotoxicity of CLP as reported by others (Bonjean *et al*., 1998; Dassonnevile *et al*., 2000). The remarkable similarity in the cytotoxic profiles of the two agents would suggest that CLP is responsible for the activity of CSE, consistent with a previous report that CLP is the major alkaloid of the root extract (Dwuma-Badu *et al*., 1978; Tachie *et al*., 1991). Interestingly, the results of the Trypan blue assay in V79 cells, which showed up to 70% viability in samples treated for 24 h at the highest concentration of CSE and CLP, contrasted sharply with the low survival rates in the clonogenic assay. This would suggest an initial cellular damage probably undetected by the Trypan blue technique but reflected in the long-term survival of the treated cells. The Trypan blue assay is based on membrane integrity, but dying (apoptotic) cells could maintain their membrane integrity for most of the apoptotic process. Such cells would essentially exclude Trypan blue but are not necessarily able to survive, proliferate, and generate colonies. This could account for the low survival rates recorded in the long-term clonogenic assay but higher viability in the Trypan blue test.

It was to investigate the circumstances of this delayed cell death that we sought to determine the cycle distribution of

TABLE 1
Toxicity of CSE and CLP to Selected Cell Lines

	IC_{50}	
Cell line	CSE (μg/ml)	CLP (μM)
V79-MZ	38.8 $\pm$ 6.20	2.1 $\pm$ 0.11
HCT-116	20.8 $\pm$ 3.38	0.7 $\pm$ 0.02
SKOV3	23.2 $\pm$ 1.06	0.9 $\pm$ 0.04
MCF7	13.9 $\pm$ 0.72	1.0 $\pm$ 0.04
MDA MB 361	68.8 $\pm$ 6.92	4.7 $\pm$ 0.48

Note. The organ specificity of the cell lines is described in Materials and Methods. Cells were treated with CSE or CLP for 24 h, and then were seeded at 100 cells/well in 6-well plates in a drug-free medium. After incubation at 37°C for 7 days, colonies of more than 50 cells were scored as survivors. Cell survival was expressed as a percentage of appropriate vehicle-treated controls. IC_{50} values (mean $\pm$ SD) were interpolated from survival curves obtained from 3 separate experiments.

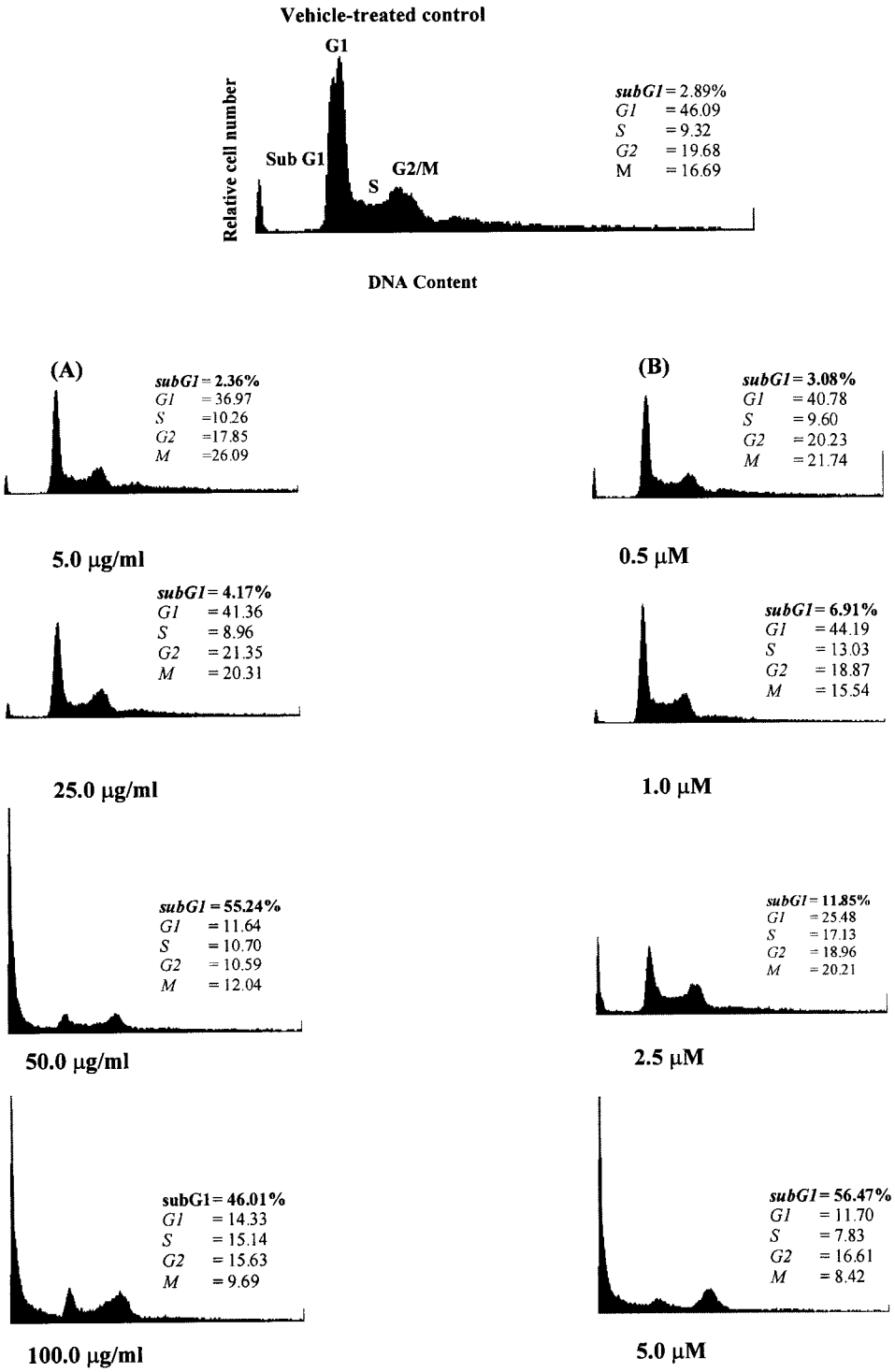

FIG. 4. The effect of CSE (A graphs) and CLP (B graphs) on the cell cycle distribution of V79 cells treated for 24 h, compared to vehicle-treated control. Cells were harvested immediately after treatment and were fixed and analyzed with the FACScan flow cytometer, as described in Materials and Methods. Histograms are representatives of 3 separate experiments.

treated cells by flow cytometry. It is intriguing that in the flow cytometry analysis, the large sub-G1 population (up to 55%) observed in samples treated for 24 h indicated that not all the dying cells were detected in the Trypan blue test. The appearance of cells in a sub-G1 population has been considered as a marker of apoptotic cell death (Darzynkiewicz *et al.*, 1992); thus our flow cytometry study would suggest that CSE and CLP induce apoptosis in V79 cells as reported previously for CLP in HL-60 leukemia cells (Dassonneville *et al.*, 2000). Many DNA-damaging agents, including mutagens and carcinogens, cause cell-cycle arrest at G1, S-phase, and G2 checkpoints, which may be followed by apoptotic cell death (Barry

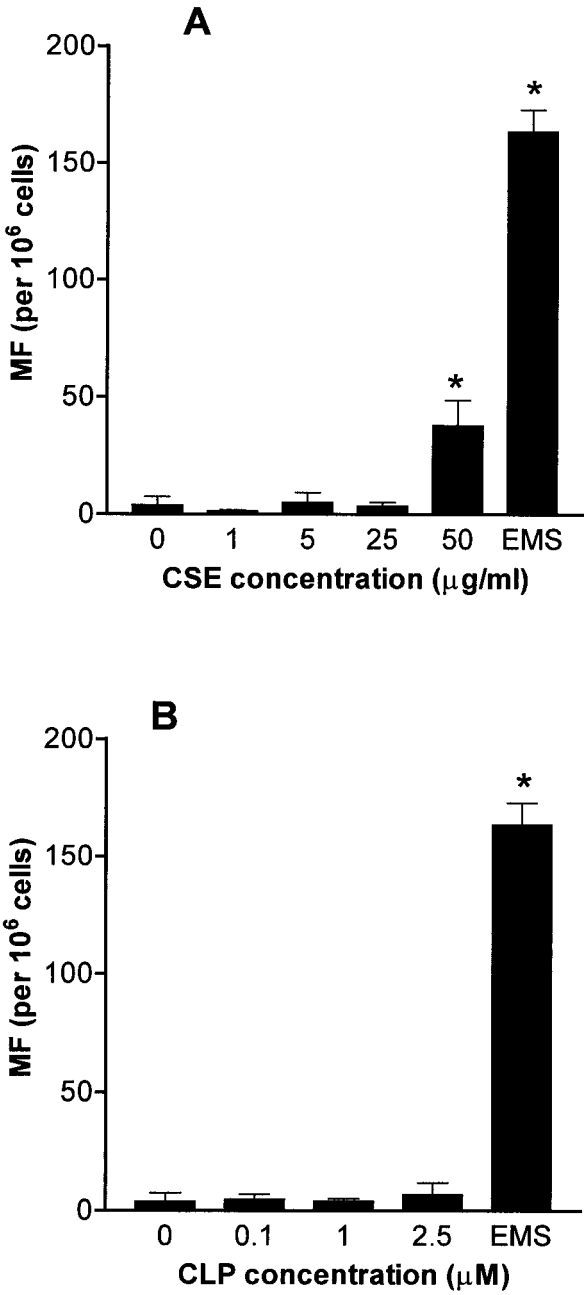

FIG. 5. *Hprt* gene mutation frequency induced by CSE (A) and CLP (B) in V79 cells treated for 24 h. Treated cells were cultured for 7 days in drug-free medium before they were moved to selective medium for the colony formation assay. The points represent mean values obtained from 3 separate experiments, each in triplicate, and are corrected for cell survival. Bars are standard deviations. *Significantly different compared to control; $p < 0.01$.

et al., 1990; Hartwell and Kastan, 1994), depending on the intensity of the damage and the repair machinery at the disposal of the cell. The cell cycle checkpoints allow sufficient time for DNA repair, yet apoptosis may ensue to eliminate overwhelmed DNA-damaged cells (Lowe and Lin, 2000; Offer *et al*., 2002). Interestingly, in our experiments the sub-G1 population increased in the absence of any obvious block in G1, S, or G2/M phases. The p53 status of a cell is an important determinant of cell cycle arrest (Diller *et al*., 1990; Kuerbitz *et al*., 1992; Livingstone *et al*., 1992). It induces cell cycle arrest at the G1/S DNA damage checkpoint through the transcriptional activation of the CDK inhibitor $p21^{Cip1/WAF1}$ (Brugarolas *et al*., 1995; El-Diery *et al*., 1993). Recent evidence also suggests that p53 and p21 are required for maintaining a G2 arrest following DNA damage (Flatt *et al*., 2000). The V79 cells used in the current study are reported to have a mutated, nonfunctional p53 (Chaung *et al*., 1997). This might have contributed to the inability of the cells to evoke a distinct arrest at any phase of the cycle.

Our data not only indicate toxicity of the agents to V79, a typical mammalian cell line, but several organ-specific human cancer cell lines. The reason for the differential sensitivity of the cell lines is not immediately clear from the studies, but the cell type and the balance of expression of apoptotic and anti-apoptotic proteins in the individual cell lines could contribute at least in part to the differential sensitivity. It is, however, noteworthy that both agents inhibited colony formation, a major factor for anticancer agents. If this property were to occur *in vivo*, our results would suggest that CSE and CLP could be used against human cancers.

One of the major problems associated with anticancer agents is the possibility of genotoxicity resulting from DNA interaction. The results of the *hprt* mutation assay would appear to indicate that CLP is not mutagenic at concentrations that invoke substantial cytotoxicity. The significant mutation frequency observed at the highest concentration of CSE could suggest the presence of a minor component, which is probably more mutagenic than CLP, the activity of which becomes apparent at high concentrations of CSE administration. Several minor alkaloids have been isolated from the plant (Paulo *et al*., 1994) whose biological activities have yet to be determined. The reason for the poor mutagenic action of CLP in the face of the reported DNA intercalation (Bonjean *et al*., 1998; Lisgarten *et al*., 2002) could be due to its topoisomerase-II inhibitory properties. Topoisomerase-II inhibition is manifest as fragmented DNA. This is a clastogenic event resulting in large deletions, which are poorly detectable in the *hprt* assay system (Kulling and Metzler, 1997) since their lethality frequently occurs through effects on neighboring essential genes (Moore *et al*., 1989). The *hprt* gene locus is known to be rich in GC sites, and CLP has been demonstrated to have high affinity for GC-rich regions of DNA (Bonjean *et al*., 1998). CLP binding and consequent damage to the *hprt* gene itself could be the reason for the inability of CLP to induce *hprt* mutants. The profound cytotoxicity of CLP probably precedes the phenotypic expression of induced mutant clones. Thus, alternative genotoxicity assessment is warranted, perhaps using an autosomal locus target as well as clastogenicity assays.

Overall, our studies indicate that CSE and CLP are potently cytotoxic to V79 cells and a number of organ-specific human

cancer cell lines. The demonstration of a broad-spectrum activity on a variety of cancer cell lines, apoptotic cell death, and apparently low genotoxicity suggest that these agents may have potential as candidates for cancer chemotherapy.

ACKNOWLEDGMENT

C.A. thanks the Commonwealth Scholarships Commission for the award of a Postgraduate Scholarship.

REFERENCES

Barry, M. A., Behnke, C. A., and Eastman, A. (1990). Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins, and hyperthermia. *Biochem. Pharmacol*. **40,** 2353–2362.

Bierer, D. E., Fort, D. M., Mendez, C. D., Luo J., Imbach, P. A., Dubenko, L. G., Jolad, S. D., Gerber, R. E., Litvak, J., Lu, Q., Zhang, P., Reed, M. J., Waldeck, N., Bruening, R. C., Noamesi, B. K., Hector, R. F., Carlson, T. J., and King, S. R. (1998). Ethnobotanical-directed discovery of the antihyperglycaemic properties of cryptolepine: Its isolation from *Cryptolepis sanguinolenta*, synthesis and *in vitro* and *in vivo* activities. *J. Med. Chem*. **41,** 894–901.

Bonjean, K., De Pauw-Gillet, M. C., Defresne, M. P., Colson, P., Houssier, C., Dassonneville, L., Bailly, C., Greimers, R., Wright, C., Quentin-Leclercq, J., Tits, M., and Angenot, L. (1998). The DNA-intercalating alkaloid cryptolepine interferes with topoisomerase II and inhibits primarily DNA synthesis in B16 melanoma cells. *Biochemistry* **37,** 5136–5146.

Boye, G. L., and Ampofo, O. (1983). Proceedings of the First International Symposium on cryptolepine. Abstract No. 4. University of Science and Technology, Kumasi, Ghana.

Brugarolas, J., Chandrasekaran, C., Gordon, J. I., Beach, D., Jacks, T., and Hannon, G. J. (1995). Radiation-induced cell cycle arrest compromised by p21 deficiency. *Nature* **377,** 552–557.

Chaung, W., Mi, L.-J., and Boorstein, R. J. (1997). The p53 status of Chinese hamster V79 cells frequently used for studies on DNA damage and DNA repair. *Nucleic Acid Res*. **25,** 992–994.

Darzynkiewicz, Z., Bruno, S., Del Bino, G., Gorczyca, W., Hotz, M. A., Lassota, P., and Traganos, F. (1992). Features of apoptotic cell measured by flow cytometry. *Cytometry* **13,** 795–808.

Dassonneville, L., Lansiaux, A., Wattelet, A., Wattez, N., Mahieu, C., Van Miert, S., Pieters L., and Bailly, C. (2000). Cytotoxicity and cell cycle effects of the plant alkaloids cryptolepine and neocryptolepine: Relation to drug-induced apoptosis. *Eur. J. Pharmacol*. **409,** 9–18.

Diller, L., Kassel, J., Nelson, C. E., Gryka, M. A., Litwak, G., Gebhardt, M., Bressac, B., Ozturk, M., Baker, S. K., Volgestein, B., *et al*. (1990). p53 functions as a cell-cycle control protein in osteosarcomas. *Mol. Cell. Biol*. **10,** 5772–5781.

Dwuma-Badu, D., Ayim, J. S., Fiagbe, N. I., Knapp, J. E., Schiff, P. L., Jr., and Slatkin, D. J. (1978). Constituents of West African medicinal plants: XX. Quindoline from *Cryptolepis sanguinolenta*. *J. Pharm. Sci.* **67,** 433–434.

El-Diery, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993). WAF1, a potential mediator of p53 tumor suppression. *Cell* **75,** 817–825.

Fields, R. D., and Lancaster, M. V. (1993). Dual-attribute continuous monitoring of cell proliferation/cytotoxicity. *Am. Biotechnol. Lab.* **11,** 48–50.

Flatt, P. M., Tang, L. J., Scatena, C. D., Szak, S. T., and Pietenpol, J. A. (2000). p53 regulation of G(2) checkpoint is retinoblastoma protein dependent. *Mol. Cell Biol*. **20,** 4210–4223.

Hartwell, L. H., and Kastan, M. B. (1994). Cell cycle control and cancer. *Science* **266,** 1821–1828.

Kirby, G. C., Paine, A., Warhurst, D. C., Noamesi, B. K., and Philipson, J. D. (1995). *In vitro* and *in vivo* antimalarial activity of cryptolepine, a plant-derived indoquinoline. *Phytother. Res.* **9,** 359–363.

Kuerbitz, S. J., Plunkett, B. S., Walsh, W. V., and Kastan, M. B. (1992). Wild-type p53 is a cell cycle checkpoint determinant following irradiation. *Proc. Natl. Acad. Sci. U.S.A.* **89,** 7491–7495.

Kulling, S. E., and Metzler, M. (1997). Induction of micronuclei, DNA strand breaks, and *hprt* mutations in cultured Chinese hamster V79 cells by the phytoestrogen coumoestrol. *Food Chem. Toxicol*. **35,** 605–613.

Lisgarten, J. N., Coll, M., Portugal, J., Wright, C. W., and Aymami, J. (2002). The antimalarial and cytotoxic drug cryptolepine intercalates into DNA at cytosine-cytosine sites. *Nat. Struct. Biol.* **9,** 57–60.

Livingstone, L. R., White, A., Sprouse, J., Livanos, E., Jacks, T., and Tlsty, T. D. (1992). Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell* **70,** 923–935.

Lowe, S. W., and Lin, A. W. (2000). Apoptosis in cancer. *Carcinogenesis* **21,** 485–495.

Moore, M. M., Harrington-Brock, K., Doerr, C. L, Dearfield, K. L. (1989). Differential mutant quantitation at the mouse lymphoma tk and CHO *hgprt* loci. *Mutagenesis* **5,** 394–403.

Noamesi, B. K., Paine, A., Kirby, G. C., Warhurst, D. C., and Philipson, G. D. (1991). *In vitro* antimalarial action of cryptolepine, an indoquinoline. *Trans. R. Soc. Trop. Med. Hyg*. **85,** 315.

Offer, H., Erez, N., Zurer, I., Tang, X., Milyavsky, M., Goldfinger, N., and Rotter, V. (2002). The onset of p53-dependent DNA repair or apoptosis is determined by the level of accumulated damaged DNA. *Carcinogenesis* **23,** 1025–1032.

Paulo, A., Duarte, A., and Gomes, E. T. (1994). *In vitro* antibacterial screening of *Cryptolepis sanguinolenta* alkaloids. *J. Ethnopharmacol*. **44,** 127–130

Tachie, A.N., Sharaf, M. H. N, Schiff, P. L., Boye, G. L., Crouch, R. C., and Martin, G. E. (1991). Assignment of the proton and carbon NMR spectra of the indoquinoline alkaloid cryptolepine. *J. Heterocycl. Chem.* **28,** 1429–1435.

Thacker, J., and Ganesh, A. N. (1989). Molecular analysis of spontaneous and ethyl methanesulphonate-induced mutations of the *hprt* gene in hamster cells. *Mutat. Res.* **210,** 103–112.

Wright, C. W., Philipson, J. D., Awe, S. O., Kirby, G. C., Warhurst, D. C., Quentin-Leclercq, J., and Angenot, L. (1996). Antimalarial action of cryptolepine and some other anhydronium bases. *Phytother. Res*. **10,** 361–363.

Yadollahi-Farsani, M., Gooderham, N. J., Davies, D. S., and Boobis, A. R. (1996). Mutational spectra of the dietary carcinogen 2-amino-1-methyl6-phenylimidazo[4,5-b]pyridine (PhIP) at the Chinese hamsters *hprt* locus. *Carcinogenesis* **17,** 617–624.