Journal of the Iowa Academy of Science: JIAS

Volume 119 | Number 1-4 Article 6

2012

Effects of Berberine, Chelerythrine, and Sanguinarine on Proliferation in Four Human Immortalized Cell Lines

David S. Senchina
Drake University

Nisarg B. Shah
Drake University

Marc G. Busch
Drake University

Jour. Iowa Acad. Sci. 119(1–4):22–27, 2012

Effects of Berberine, Chelerythrine, and Sanguinarine on Proliferation in Four Human Immortalized Cell Lines

DAVID S. SENCHINA[1,*], NISARG B. SHAH[2] and MARC G. BUSCH[1]

[1]Department of Biology, Drake University, Des Moines, IA
[2]Pharmacy Program, Drake University, Des Moines, IA

Bloodroot (*Sanguinaria canadensis* L., Papaveraceae) is a plant rich in benzophenanthridine (isoquinoline) alkaloids such as sanguinarine and chelerythrine. Both isolated alkaloids and whole-tissue rhizome extracts have demonstrated *in vitro* anticarcinogenic (antiproliferative) activity in limited immortalized cell culture models, but the relative contribution of various alkaloid constituents to whole-tissue extract activity, and whether or not various alkaloids may act synergistically, has not been investigated. We challenged four immortalized cell lines (Jurkat, K562, Ramos, U937) with various doses of chelerythrine, sanguinarine, and berberine (a structurally and functionally similar alkaloid absent from bloodroot), alone and in all possible combinations, and measured proliferation rates 48 hours post-treatment. K562 cells were unaffected by all doses of berberine and chelerythrine tested, but showed antiproliferative activity at 0.5 μg/mL sanguinarine. The doses of sanguinarine necessary to elicit anti-proliferative effects in these experiments matched well with those seen in whole-tissue rhizome extracts demonstrating similar anti-proliferative effects. When a 2 μg/mL dose of sanguinarine was tested in all four cell lines, sanguinarine elicited even stronger antiproliferative effects in Jurkat, Ramos, and U937 cells compared to K562 cells; these three cell lines also demonstrated reduced proliferation in the presence of chelerythrine at a dose of 1 μg/mL whereas K562 did not. At the doses tested here, berberine had no direct effect on proliferation. These results suggest that sanguinarine may be responsible for the bulk of anticarcinogenic activity from bloodroot whole-tissue rhizome extracts, but that other alkaloids such as chelerythrine may also play important roles contingent on cell type.

INTRODUCTION

Bloodroot (*Sanguinaria canadensis* L.) is an herbaceous perennial found in eastern and central North America (Graf et al., 2007; Figure 1). Botanically, it is the only member of the genus *Sanguinaria*. This genus belongs to the poppy family (Papaveraceae) which is well-known for its medicinal compounds. The Native American tradition of using bloodroot as a medicinal plant has changed but continued into the twenty-first century where it has had two primary indications: as a dental care agent (Hong et al. 2005) and an anti-skin cancer agent (Moran and Helm 2008; DeStefano et al. 2009; Wang and Warshaw 2012). More recent research suggests it may be anticarcinogenic against other non-dermal cells types, such as breast, intestinal, and lymphatic cancer cells (Han et al. 2008; Kim et al. 2008; Choi et al. 2009a; Mazzio and Soliman 2009; Lee et al. 2012).

The purported primary bioactive molecule from bloodroot is sanguinarine, a benzophenanthridine (isoquinoline) alkaloid which is the most prevalent alkaloid in bloodroot extracts (Campbell et al. 2007; De Stefano et al. 2009). It is also found in other members of Papaveraceae as well as the fumitory family (Fumariaceae), the buttercup family (Ranunculaceae), and the citrus family (Rutaceae) (Vecera et al. 2007). Most research has focused on the activity of this lone molecule in *in vitro* cell culture models (Choi et al. 2009b; Mishra et al. 2009; Vrba et al. 2009) where it has conclusively shown anticarcinogenic effects that appear to be specific towards cancerous cells and not healthy cells (Ahmad et al. 2000). Our team has shown similar activity by whole-tissue extracts from bloodroot rhizomes, in which sanguinarine was the most abundant alkaloid; in our model, those extracts reduced proliferation of the human myelogenous leukemia cell line K562 while stimulating proliferation of peripheral blood mononuclear cells from healthy young human adults (Senchina et al. 2009a). However, formal analyses of the proliferation data (and accompanying cytokine secretion data) from that and other studies (Senchina et al. 2009b; Senchina et al. 2012) collectively suggest that sanguinarine content alone may not account for all observed immunoodulatory effects. Whether or not other alkaloids present in the plant (or other structurally-similar compounds found in other plant genera) may contribute to such effects is unknown. Chelerythrine is one such candidate alkaloid also found in bloodroot (Graf et al. 2007), other members of Papaeveraceae, and two other families (Fumariaceae and Rutaceae; Zdařilova et al. 2006). Chelerythrine demonstrates similar anti-cancer properties as sanguinarine (Vogler et al. 2009; Hammerová et al. 2011). Whether alkaloids such as chelerythrine and sanguinarine may interact in a synergistic or combinatorial manner has not been directly demonstrated; however, Larsson et al. (2009) showed that sanguinarine weakly synergizes with other chemotherapeutic drugs *in vitro*.

As an attempt to "bridge the gap" between those studies that examined cell proliferation or apoptosis utilizing alkaloid-rich bloodroot extracts and those using sanguinarine alone, a series of experiments were undertaken. The anti-proliferative activity of both sanguinarine and chelerythrine were investigated, as well as a structurally-similar third alkaloid (berberine) which is not present in bloodroot but is found in other closely related plant families. Four immortalized cell lines representative of the human immune system (Jurkat, K562, Ramos, U937) were tested against the three alkaloids. The aim of the first experiment was to determine if the three alkaloids had similar or different effects on

* Author for correspondence (DSS): Olin Hall 415, 2507 University Ave., Drake University, Des Moines, IA 50311, Tel. 1 515 271 2956, Fax 1 515 271 3702, dssenchina@drake.edu

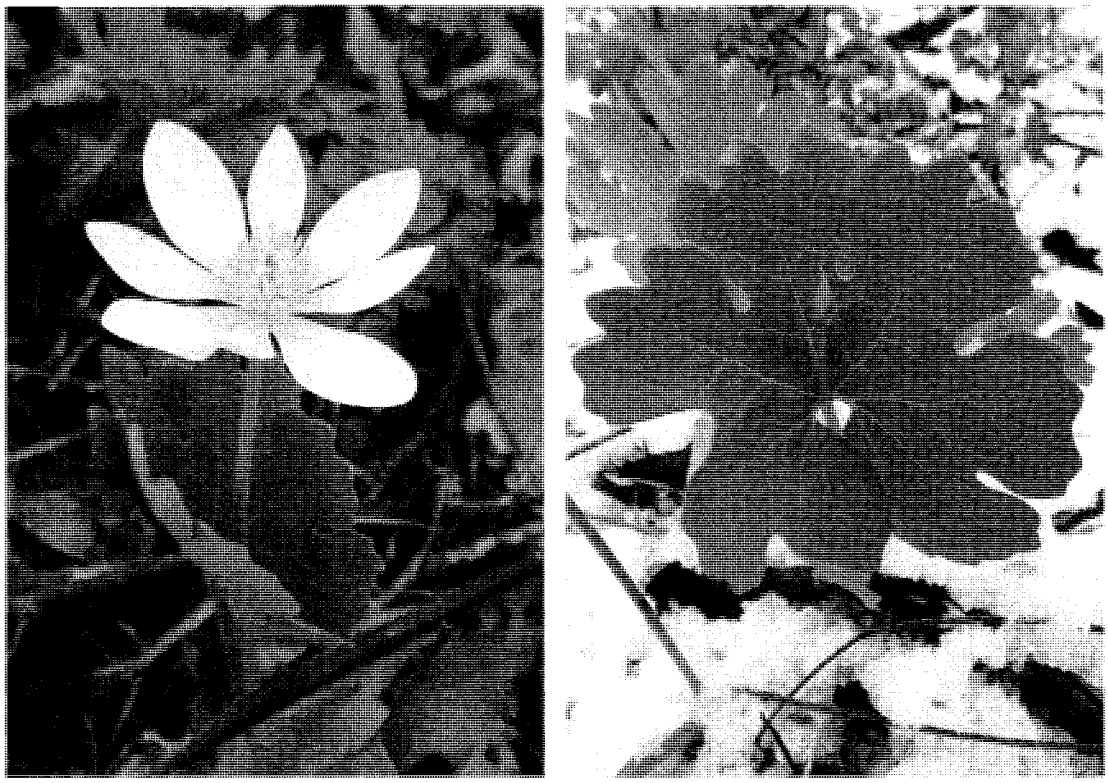

Fig. 1. Bloodroot (*Sanguinaria canadensis* L.) from Carr Woods in Ames, Iowa, spring 2013. Left: Plant in anthesis with newly-emerged leaves clasping the petiole, approximately 9 cm tall (April). Right: Plant fruiting (see leaf center) with more mature leaves, after a late snowstorm (May).

K562 cell proliferation at various doses including those found in whole-tissue rhizome extracts; an ancillary aim of the first experiment was to determine if the alkaloids acted synergistically. The aim of the second experiment was to determine if other cell lines responded similarly to K562 cells under the same treatment doses. For both experimental series, cells were cultured with each alkaloid, alone or in all possible combinations, for 48 hours after which time cells were counted and compared to baseline.

METHODS

K562 cells (representing undifferentiated granulocytes) were generously provided by Dr. Joan E. Cunnick (Iowa State University). Jurkat E6-1 (representing T cells), Ramos RA1 (representing B cells), and U937 (representing monocytes) cell lines were purchased from ATCC. All four cell lines are non-adherent in culture. The culture media was CRPMI, based on a recipe of 440 mL RPMI (GIBCO) supplemented with 10 mL of penicillin/streptomycin (GIBCO) and 50 mL fetal bovine serum (Mediatech). Berberine, chelerythrine, and sanguinarine (Figure 2; obtained from Sigma-Aldrich) were diluted into stock concentrations (see below), stored at -80°C, and tested within two months of preparation. Some of these compounds are photosensitive and require being kept in dark conditions; however, aqueous solutions of dilutions stored at -80°C are biochemically stable and maintain immunomodulatory activity with long-term light-protected storage at -80°C (Senchina et al. 2009b).

Cell cultures were grown for each of the four cell lines such that approximately 150 mL of cell culture at 1.0×10^5 cells/mL was established prior to testing. For the experiments, cells were partitioned as 5 mL cultures, with three flasks established for each of eight different treatment conditions: (1) control (media alone), (2) berberine alone, (3) chelerythrine alone, (4) sanguinarine alone, (5) berberine + chelerythrine, (6) berberine + sanguinarine, (7) chelerythrine + sanguinarine, (8) and all thee alkaloids together. The initial concentrations of sanguinarine and chelerythrine for stock solutions were chosen based on average concentrations found in several batches of rhizome extracts, including those identified previously (Senchina et al. 2009a) and additional rhizome extracts currently under experimentation: sanguinarine at 0.8 μg/mL and chelerythrine at 0.4 μg/mL. For berberine, we arbitrarily chose to test it at the same concentration as chelerythrine. Final concentrations of each alkaloid in the wells at the 1× concentration were as follows: 0.01 μg/mL for both berberine and chelerythrine, and 0.02 μg/mL for sanguinarine. Cells were variously treated with 1×, 10×, or 100× doses. Cultures were grown under standard incubation conditions (37°C, 5% CO_2, humidified) for 48 hours. After incubation, number and viability of cells post-treatment were assessed manually using a hemacytometer and Trypan blue staining. Results were averaged across the three replicates for all eight conditions.

One-way ANOVA was used to check for significant differences in proliferation across treatment conditions (PASW 17.0, SPSS Inc.); when a main effect of treatment was uncovered, significance between pairwise comparisons was determined *post-hoc* using LSD. Significance was defined as $\alpha<0.05$ whereas a trend towards significance was defined as $0.05<\alpha<0.1$. When multiple comparisons were used within an ANOVA, a correction was made by dividing the starting α-value of 0.05 by the number of comparisons and redefining significance based on the corrected p-value.

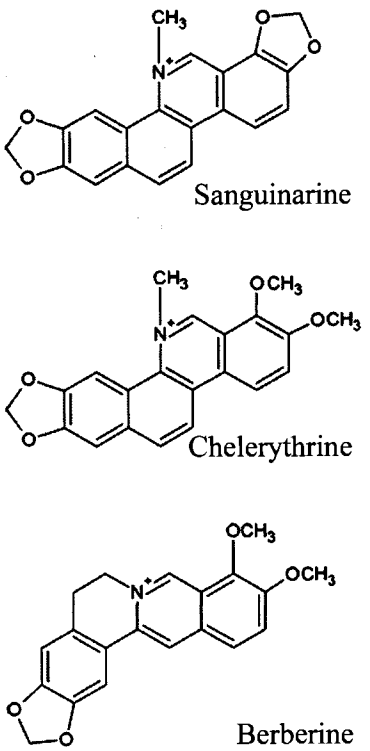

Fig. 2. Benzophenanthridine alkaloids used in this study. According to Chaturvedi et al. (1997), all three are considered chemical analogues of each other, and chelerythrine and sanguinarine are considered structural homologues. Sanguinarine and chelerythrine are produced by bloodroot whereas berberine is not. Structures were drawn using ChemSketch (ACD/Labs).

RESULTS

To determine the concentration of alkaloids necessary to influence cell proliferation, initially only the K562 cells were tested at 1× (representing those found in rhizome ethanol extracts based on Senchina et al. [2009a]), 10×, and 100× concentrations (Table 1). There was a main effect of treatment ($p<0.001$). Across all doses tested, only sanguinarine was able to significantly diminish cell proliferation as compared to control, and only at the 100× concentration (2.0 μg/mL). This effect was significant when sanguinarine was used alone or in combination with one or both other alkaloids ($p<0.001$ all cases). There was also a trend for the combination of berberine and chelerythrine to diminish proliferation at 100× concentrations (1.0 μg/mL each; $p=0.001$).

To better understand the dose-response relationship between sanguinarine and K562 proliferation, sanguinarine alone was tested at 25×, 50×, 75×, and 100× concentrations (Figure 3). There was a main effect of treatment ($p<0.001$) such that proliferation was significantly lower at all treatment doses compared to the vehicle control (all $p<0.001$), with the 25× dose being 0.5 μg/mL. The collective data demonstrated the expected dose-response relationship (e.g., average proliferation values decreased as the concentration of sanguinarine increased). However, there were no significant differences when the various treatments were compared against each other.

To determine if other cell lines responded to the three alkaloids similarly to how K562 cells had, cultures of Jurkat, Ramos, and U937 cells were tested in the same manner using only the 100× alkaloid concentrations. Although all the results from all three cell lines are presented together in Table 2, separate ANOVA were run for each cell type independently of others. There was a main effect of treatment for all three cell lines (all $p<0.001$); p-values presented in the remainder of this paragraph reference comparisons to control. Berberine alone had no effect on cell proliferation in any of the three cell lines. Chelerythrine alone or combined with berberine diminished cell proliferation in all three cell lines ($p\leq0.003$). Sanguinarine alone caused complete cell culture death in all three cell lines ($p\leq0.001$). Interestingly, the effects of sanguinarine on proliferation were sometimes different when sanguinarine was used in combination with other alkaloids, and this phenomenon varied by cell type. In the case of Jurkat and U937 cells, sanguinarine combined with one or both other alkaloids either resulted in complete cell culture death or cell culture stasis ($p\leq0.001$). The results with Ramos cells were more complex. Compared to control, the proliferative response of Ramos cells was significantly lower when sanguinarine was administered with one or both other alkaloids ($p<0.001$). However, administration of sanguinarine in combination with one or both other alkaloids did not result in complete cell culture death, as was the case when sanguinarine was administered alone.

Table 1. Proliferative response of K562 cells to three alkaloids at 1×, 10×, and 100× concentrations (starting concentrations of 0.4 μg/mL for berberine and chelerythrine, and 0.8 μg/mL for sanguinarine). Each experiment was performed in triplicate. Values are cell counts (expressed as 1.0×10^5 cells/mL) ± standard error. Asterisks (*) indicate statistically significant differences of treatment compared to control within the same column (all $p<0.001$).

	Relative Concentration		
	1×	10×	100×
Control	14.9 ± 1.0	14.9 ± 1.0	14.9 ± 1.0
Berberine	14.9 ± 1.2	13.4 ± 1.5	16.6 ± 0.8
Chelerythrine	17.0 ± 0.9	18.1 ± 2.4	16.0 ± 1.1
Sanguinarine	14.4 ± 1.5	15.8 ± 3.5	1.8 ± 0.2*
Berberine + Chelerythrine	14.8 ± 0.3	15.4 ± 0.5	14.5 ± 1.0
Berberine + Sanguinarine	13.9 ± 0.8	12.7 ± 0.2	3.4 ± 0.5*
Chelerythrine + Sanguinarine	15.0 ± 0.9	15.5 ± 0.7	2.4 ± 0.3*
All 3 Alkaloids	11.2 ± 0.8	11.7 ± 1.3	3.6 ± 0.4*

Table 2. Proliferative response of Jurkat, Ramos, and U937 cells to three alkaloids at 100× concentrations. Each experiment was performed in triplicate. Values are cell counts (expressed as 1.0×10^5 cells/mL) ± standard error. BL=below limits of detection. The dagger (†) indicates data from Table 1 (100× concentration) included again here for comparison. Asterisks (*) indicate statistically significant differences of treatment compared to control within the same column (all $p \leq 0.003$).

	Cell Line			
	Jurkat	K562†	Ramos	U937
Control	30.7 ± 7.6	14.9 ± 1.0	27.0 ± 6.5	21.3 ± 4.9
Berberine	23.3 ± 3.5	16.6 ± 0.8	27.3 ± 4.7	24.7 ± 8.3
Chelerythrine	1.3 ± 0.8*	16.0 ± 1.1	5.0 ± 1.3*	3.7 ± 2.7*
Sanguinarine	BL*	1.8 ± 0.2*	BL*	BL*
Berberine + Chelerythrine	1.3 ± 0.8*	14.5 ± 1.0	6.3 ± 0.3*	2.7 ± 1.2*
Berberine + Sanguinarine	1.0 ± 0*	3.4 ± 0.5*	2.0 ± 1.5*	BL*
Chelerythrine + Sanguinarine	BL*	2.4 ± 0.3*	2.3 ± 1.2*	BL*
All 3 Alkaloids	BL*	3.6 ± 0.4*	2.0 ± 0.6*	BL*

DISCUSSION

The aims of this experimental series were threefold: (1) to compare the effects of berberine, chelerythrine, and sanguinarine, alone and in all possible combinations and in various doses, on proliferation of K562 cells; (2) to characterize the response of other immortalized cell lines relative to K562 cells; and (3) to determine if alkaloids act synergistically to influence proliferation. Findings from these experiments confirmed and extended previous work from our lab and others.

To address aim #1, the effects of various doses of all three alkaloids on K562 cell proliferation was determined (Table 1 and Figure 3). K562 cell proliferation was negatively influenced by sanguinarine but not berberine or chelerythrine. Sanguinarine concentrations between 0.5−2.0 μg/mL were necessary to observe statistically significant reductions in cell proliferation, whereas concentrations of 0.2 μg/mL or less had no effect on proliferation. Previously, our lab demonstrated that whole-tissue extracts from bloodroot rhizomes were able to diminish proliferation of K562 cells yet stimulate proliferation of peripheral blood mononuclear cells obtained from healthy young human adults, and that sanguinarine was the principal component of those extracts (Senchina et al. 2009a). In that previous study, a cold water extract negligibly influenced K562 proliferation, whereas a hot water extract reduced the cell population to 11% of the control value, and an ethanol extract resulted in complete cell culture death. Sanguinarine concentrations for the three extracts once diluted in cell culture were 0.17, 1.09, and 2.93, μg/mL, respectively. Thus, the sanguinarine content of the hot water and ethanol rhizome extracts used previously fall within the 0.5−2.0 μg/mL range determined in this experiment to be necessary to see effects. The 50× dose of sanguinarine (1.0 μg/mL) is most similar to the hot water extract used previously; notably, the 50× dose of sanguinarine reduced the cell population to 14% of the control value, consistent with the effect of the hot water extract. These results suggest that, at least in the K562 model, sanguinarine alone may explain the anti-proliferative effect seen from whole-tissue rhizome extracts.

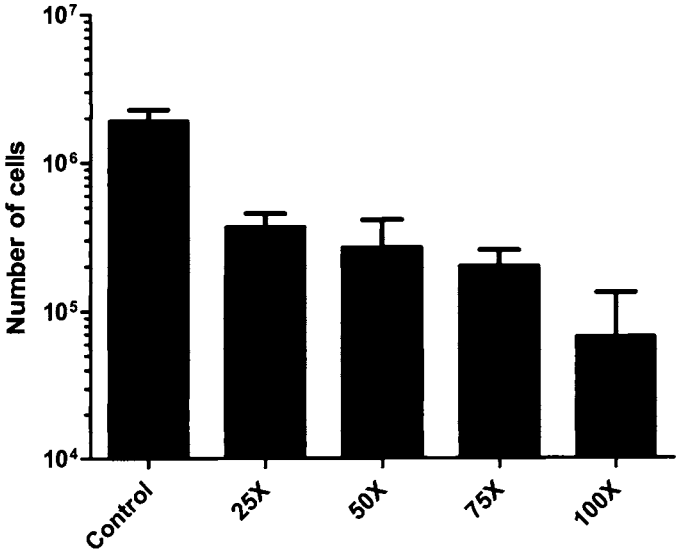

Fig. 3. Proliferative response of K562 cells to sanguinarine in a dose-response test. Each experiment was performed in triplicate. Values are cell counts (expressed as cells/mL) ± standard error.

To address aim #2, the effects of the 100× concentrations of all three alkaloids, alone or in various combinations, on proliferation of Jurkat, Ramos, and U937 cells was ascertained (Table 2). Compared to K562 cells, other immortalized cell lines may be more susceptible to the anti-proliferative effects of alkaloids. Once again, berberine had no effect in any of the cell lines. Chelerythrine (1.0 μg/mL in the 100 × dose) negatively impacted proliferation in all three cell lines (unlike in K562 cells). Sanguinarine demonstrated an even more pronounced anti-proliferative effect than in K562 cells. Sanguinarine combined with one or more other alkaloids resulted in complete cell culture death or cell culture stasis in both Jurkat and U937 cells; by contrast, in Ramos cells sanguinarine showed a stronger anti-proliferative effect when it was used alone, and a lesser effect when it was combined with other alkaloids.

Why Ramos and K562 cells are less sensitive to and Jurkat and U937 cells are more sensitive to the effects of sanguinarine, and why Jurkat, Ramos, and U937 cells are more sensitive to chelerythrine than K562 cells is not immediately clear. Immortalized cells are indefinitely replicating cancerous cells derived clinically; each cell line originally started as normal human immune cells. Hematopoetically, Jurkat and Ramos cells (originally T and B cells, respectively) derive from the common lymphoid progenitor whereas K562 and U937 cells (originally undifferentiated granulocytes and monocytes, respectively) derive

from the common myeloid progenitor. Thus, there is nothing in the hematopoetic lineages of these cell lines that suggests a developmental relationship.

Berberine, chelerythrine, and sanguinarine are structurally similar (Figure 2). Both chelerythrine and sanguinarine are produced by bloodroot whereas berberine is not; notably, both chelerythrine and sanguinarine demonstrated anti-proliferative activity whereas berberine did not. Berberine was chosen as a comparison alkaloid because it is structurally similar to bloodroot alkaloids and produced naturally by other plant taxa, including other members of the poppy family (Israilov et al. 1986; Takemura et al. 2010) as well as eight other plant families (Philogène et al. 1984). However, berberine has shown anticancer activity in alternative models including K562 cells (Pazhang et al. 2011) and U937 cells (Jantova et al. 2007). Differences between our work and others' are likely due to variances in experimental models chosen, but it is important to note that since berberine does not naturally occur in bloodroot, it is perhaps not surprising to see that it behaved differently than bloodroot alkaloids in our model.

To address aim #3, alkaloids were tested alone and in various combinations in all four cell lines (Table 2). No synergistic effects were apparent in our investigation in any of the examined cell lines; however, this may be an artifact of the experimental design and complex interplay between different alkaloids or cell types. For example, in Jurkat and U937 cells, sanguinarine showed such strong effects across all conditions that it possibly "masked" or "overrode" any interplay between it and chelerythrine. By contrast, chelerythrine appeared to diminish the anti-proliferative effects of sanguinarine against K562 and Ramos cells. The difference in sanguinarine-treated wells versus wells treated with one or more other alkaloids were not statistically significant in either the K562 or Ramos cells at 100× concentrations after correcting the α value for multiple comparisons. Given the structural similarity between the three alkaloids, it is possible that chelerythrine and sanguinarine bind to the same cell surface receptors and may compete with each other for docking sites. It is conceivable that if chelerythrine outcompetes sanguinarine for binding sights yet has less anticarcinogenic activity, it could diminish the effects of sanguinarine when the two are administered simultaneously in culture. Differences seen across the different cell lines may be explained by differences in presence/absence, quantity, or timing of expression of those receptors between the cell types studied here. Further experiments are necessary to clarify interactions between alkaloids and the exact identity of the alkaloid receptor.

The clinical utility of bloodroot alkaloids against cancer is reaffirmed by these results, as both sanguinarine and chelerythrine demonstrated anti-proliferative activity against two or more cell lines (and has been demonstrated previously in other cell lines). To the best of our knowledge, this is the first report of the effects of: berberine on Jurkat and Ramos cells; chelerythrine on K562, Ramos, and U937 cells; and sanguinarine on Jurkat, K562, and Ramos cells. Reports elsewhere have addressed the effects of berberine on K652 (Pazhang et al. 2011) and U937 (Jantova et al. 2007) cells, chelerythrine on Jurkat cells (Vogler et al. 2009), and sanguinarine on U937 cells (Han et al. 2008).

This study has several limitations. Only limited doses were used, and it is possible that higher or lower doses would elicit alternate results. We examined only three alkaloids, and only two of which were present in bloodroot; although several other bloodroot alkaloids have been characterized (and more are likely to be discovered), they were not readily commercially available at the time of the study. Our study examined only immortalized cell lines representative of the human immune system, so it is unknown how generalizable these results are to other somatic cell types or primary cells.

Altogether, these results suggest that benzophenanthridine alkaloids may impact on cancer cell proliferation in a complex manner contingent on cancer cell type, dose, and presence/absence of other alkaloids (and possibly other phytochemicals). Of the three alkaloids, sanguinarine demonstrated the strongest anti-proliferative effect whereas berberine demonstrated none, and chelerythrine elicited heterogeneous effects contingent on cell type tested. While our findings should strongly encourage medical scientists to continue their investigations of bloodroot extracts and alkaloids in cancer models, caution is also necessary as some adverse effects have been reported (Cienki and Zarat 2010; Childress et al. 2011; Vlachojannis et al. 2012). Thus, it is imperative that scientists redouble their efforts at educating medical practitioners and the lay public alike as to both the potential clinical advantages and potential clinical adverse effects of bloodroot extracts or constituent alkaloids.

ACKNOWLEDGMENTS

This study was funded by Iowa Academy of Sciences grant #10-04 awarded to DSS. All three authors contributed experimental data. Structures and figures were produced by MGB and DSS. DSS wrote the paper and took the photographs.

REFERENCES

AHMAD, N., S. GUPTA, M. M. HUSAIN, K. M. HEISKANEN, and H. MUKHTAR. 2000. Differential antiproliferative and apoptotic response of sanguinarine for cancer cells versus normal cells. Clinical Cancer Research 6:1524–1528.

CAMPBELL, S., J. AFFOLTER, and W. RANDLE. 2007. Spatial and temporal distribution of the alkaloid sanguinarine in *Sanguinaria canadensis* L. (Bloodroot). Economic Botany 61:223–234.

CHATURVEDI, M. M., A. KUMAR, B. G. DARNAY, G. B. N. CHAINY, S. AGARWAL, and B. B. AGARWAL. 1997. Sanguinarine (pseudochelerythrine) is a potent inhibitor of NF-κB activation, IκBα phosphorylaytion, and degradation. Journal of Biological Chemistry 272:30129–30134.

CHILDRESS, M. O., R. C. F. BURGESS, C. H. HOLLAND, and H. R. GELB. 2011. Consequences of intratumoral injection of a herbal preparation containing blood root (*Sanguinaria canadensis*) extract in two dogs. Journal of the American Veterinary Medical Association 239:374–379.

CHOI, W. Y., C. Y. JIN, M. H. HAN, G. Y. KIM, N. D. KIM, W. H. LEE, S. K. KIM, and Y. H. CHOI. 2009a. Sanguinarine sensitizes human gastric adenocarcinoma AGS cells to TRAIL-mediated apoptosis via down-regulation of AKT and activation of caspase-3. Anticancer Research 29:4457–4465.

CHOI, Y. H., W. Y. CHO, S. H. HONG, S. O. KIM, G.-Y. KIM, W. H. LEE, and Y. H. YOO. 2009b. Anti-invasive activity of sanguinarine through modulation of tight junctions and matrix metalloproteinase activities in MDA-MB-231 human breast carcinoma cells. Chemico-Biological Interactions 179:185–191.

CIENKI, J. J. and L. ZARET. 2010. An Internet misadventures: bloodroot salve toxicity. Journal of Alternative and Complementary Medicine 16:1125–1127.

DESTEFANO, I., G. RASPAGLIO, G. F. ZANNONI, D. TRAVAGLIA, M. G. PRISCO, M. MOSCA, C. FERLINI, G. SCAMBIA, and D. GALLO. 2009. Antiproliferative and antiangiogenic effects of the benzophenanthridine alkaloid sanguinarine in melanoma. Biochemical Pharmacology 78:1374–1381.

GRAF, T. N., K. E. LEVINE, M. E. ANDREWS, J. M. PERLMUTTER, S. J. NIELSEN, J. M. DAVIS, M. C. WANI, and N. H. OBERLIES. 2007. Variability in the yield of benzophenanthridine

alkaloids in wildcrafted vs. cultivated bloodroot (*Sanguinarina canadensis* L.) Journal of Agricultural and Food Chemistry 55:1205–1211.

HAMMEROVÁ, J., S. ULDRIJAN, E. TÁBORSKÁ, and I. SLANINOVÁ. 2011. Benzo[c]phenanthridine alkaloids exhibit strong antiproliferative activity in malignant melanoma cells regardless of their p53 status. Journal of Dermatological Science 62:22–35.

HAN, M. H., Y. H. YOO, and Y. H. CHOI. 2008. Sanguinarine-induced apoptosis in human leukemia U937 cells via Bcl-2 downregulation and caspase-3 activation. Chemotherapy 54:157–165.

HONG, S. J., S. S. JEONG, and K. B. SONG. 2005. Effects of sanguinaria in fluoride-containing dentrifices on the remineralisation of subcutaneous lesion in vitro. International Dental Journal 55:128–132.

ISRAILOV, I. A., V. A. CHELOMBIT'KO, and L. E. NAZAROVA. 1986. Argemone alkaloids. Chemistry of Natural Compounds 22:742–743.

JANTOVA, S., L. CIPAK, and S. LETASIOVA. 2007. Berberine induces apoptosis through a mitochondrial/caspase pathway in human promonocytic U937 cells. Toxicology In Vitro 21:25–31.

KIM, S., T. J. LEE, J. LEEM, K. S. CHOI, J. W. PARK, and T. K. KWON. 2008. Sanguinarine-induced apoptosis: generation of ROS, down-regulation of Bcl-2, c-FLIP, and synergy with TRAIL. Journal of Cellular Biochemistry 104:895–907.

LARSSON, D., S. HASSAN, R. LARSSON, K. OBERG, and D. GRANBERG. 2009. Combination analysis of anti-cancer drugs on human neuroendocrine tumor cell lines. Cancer Chemotherapy and Pharmacology 65:5–12.

LEE, J. S., W. K. JUNG, M. H. JEONG, T. R. YOON, and H. K. KIM. 2012. Sanguinarine induces apoptosis of HT-29 human colon cancer cells via the regulation of Bax/Bcl-2 ratio and caspase-9-dependent pathway. International Journal of Toxicology 31:70–77.

MAZZIO, E. A. and K. F. SOLIMAN. 2009. In vitro screening for the tumoricidal properties of international medicinal herbs. Phytotherapy Research 23:385–398.

MISHRA, V., D. K. SAXENA, and M. DAS. 2009. Effect of argemone oil and argemone alkaloid, sanguinarine, on Sertoli-germ cell coculture. Toxicology Letters 186:104–110.

MORAN, A. M. and K. F. HELM. 2008. Histopathologic findings and diagnostic difficulties posed with use of escharotic agents for treatment of skin lesions: a case report and review of the literature. Journal of Cutaneous Pathology 35:404–406.

PAZHANG, Y., S. AHMADIAN, M. MAHMOUDIAN, and M. SHAFIEZADEH. 2011. Berberine-induced apoptosis via decreasing the surviving protein in K562 cell line. Medical Oncology 28:1577–1583.

PHILOGÈNE, B. J. R., J. T. ARNSON, G. H. N. TOWERS, Z. ABRAMOWSKI, F. CAMPOS, D. CHAMPAGNE, and D. MCLACHLAN. 1984. Berberine: a naturally occurring phototoxic alkaloid. Journal of Chemical Ecology 10:115–123.

SENCHINA, D. S., G. N. FLINN, D. A. MCCANN, M. L. KOHUT, and C. T. SHEARN. 2009a. Bloodroot (*Sanguinaria canadensis* L., Papaveraceae) enhances proliferation and cytokine production by human peripheral blood mononuclear cells in an in vitro model. Journal of Herbs, Spices, and Medicinal Plants 15:45–65.

SENCHINA, D. S., J. E. HALLAM, A. S. DIAS, and M. A. PERERA. 2009b. Human blood mononuclear cell in vitro cytokine response before and after two different strenuous exercise bouts in the presence of bloodroot and echinacea extracts. Blood Cells, Molecules, and Diseases 43:298–303.

SENCHINA, D. S., J. E. HALLAM, N. M. THOMPSON, N. A. NGUYEN, J. R. ROBINSON, and M. A. D. N. PERERA. 2012. Alkaloids and endurance athletes. Track and Cross Country Journal 2:2–18.

TAKEMURA, T., N. IKEZAWA, K. IWASA, and F. SATO. 2010. Metabolic diversification of benzylisoquinoline alkaloid biosynthesis through the introduction of a branch pathway in *Eschscholzia californica*. Plant Cell Physiology 51:949–959.

VECERA, R., B. KLEDJUS, P. KOSINA, J. OROLIN, M. STIBOROVÁ, S. SMRCEK, J. VICAR, Z. DVORÁK, J. ULRICHOVÁ, V. KUBÁN, P. ANZENBACHER, and V. SIMÁNEK. 2007. Disposition of sanguinarine in the rat. Xenobiotica 37:549–558.

VLACHOJANNIS, C., F. MAGORA, and S. CHRUBASIK. 2012. Rise and fall of oral health products with Canadian bloodroot extract. Phytotherapy Research 26:1423–1426.

VOGLER, M., K. WEBER, D. DINSDALE, I. SCHMITZ, K. SCHULZE-OSTHOFF, M. J. DYER, and G. M. COHEN. 2009. Different forms of cell death induced by putative BCL2 inhibitors. Cell Death and Differentiation 16:1030–1039.

VRBA, J., P. DOLEZEL, J. VICAR, and J. ULRICHOVÁ. 2009. Cytotoxic activity of sanguinarine and dihydrosanguinarine in human promyelocytic leukemia HL-60 cells. Toxicology In Vitro 23:580–588.

WANG, M. Z. and E. M. WARSHAW. 2012. Bloodroot. Dermatitis 23:281–283.

ZDAŘILOVA, A., J. MALIKOVÁ, Z. DVORÁK, J. ULRICHOVÁ, and V. SIMÁNEK. 2006. Quaternary isoquinoline alkaloids sanguinarine and chelerythrine: in vitro and in vivo effects. Chemiké Listy 100:30–41.