Comparison of the Effects of Alkaloids Present in Traditional Native American Medicinal Plants on Melanoma and Breast Cancer Cell Lines

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ABSTRACT

Several studies on dietary and medicinal herbs have suggested that they might be useful tools in the chemotherapeutic treatment of cancer. While past research has focused on herbs found in traditional Chinese or Ayurvedic medicine, little if any research has been done on preparations used in traditional Native American medicine. Goldenseal (Hydrastis canadensis L.) and bloodroot (Sanguinaria canadensis L.) are two herbs that have been used by Native Americans as a treatment against inflammation, microbes, ulcers and cancer. Chemical studies conducted on these herbs have shown that the major alkaloid phytochemicals found in these plants are berberine and sanguinarine, respectively. Berberine and sanguinarine have been previously reported to inhibit cell proliferation of different types of myelogenous and squamous cell cancers. Some mechanisms by which berberine and sanguinarine may inhibit cell proliferation include acting as a topoisomerase poison and inhibiting NFκB. The purpose of this study was to compare the effects of berberine and sanguinarine on cell proliferation of two less invasive (M10 and MCF-7) and two highly invasive (M2 and 231) melanoma and breast cancer cell lines, respectively, and to describe their effects on DNA synthesis and localization of cyclin D1. Experiments were conducted using the cell proliferation assay sulforhodamine B (SRB) on cells treated with phytochemical to quantify inhibition in growth. Immunolabeling experiments with treated cells labeled with bromodeoxyuridine (BrdU) and antibodies to proliferating cell nuclear antigen (PCNA) visualized effects on DNA synthesis; labeling with cyclin D1 revealed changes in the distribution of this cell cycle regulatory protein within the cells. Results demonstrate that 231 breast cancer cells were more sensitive to berberine than the other cell lines. Also, sanguinarine was more toxic to cells than berberine with slightly more sensitivity towards M2 melanoma and approximately equal efficacy among the other cell lines. Immunolabeling experiments show that DNA synthesis and cyclin D1 were not inhibited by berberine in M10 or MCF-7 cells. Sanguinarine, however, inhibited DNA
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synthesis in M10 and MCF-7 cells. Interestingly, cyclin $D_1$ was not altered in M10 cells, but was completely absent from the nucleus in MCF-7 cells. M2 and 231 cells appeared apoptotic after treatment with berberine or sanguinarine. This study found, that although structurally similar, berberine and sanguinarine have different toxicities and effects on the cell cycle in these melanoma and breast cancer cell lines. These studies also suggest that neither phytochemical is specific to invasiveness, but their effects vary among each cell line. Further research exploring the effects of these phytochemicals on other types of cancer cell lines and non-cancerous cell lines should be done. In addition, experiments identifying the substituents of sanguinarine that cause a redistribution of cyclin $D_1$ in MCF-7 cells should be conducted, as well as additional experiments determining why 231 cells are more sensitive to berberine.

Introduction

Numerous studies on dietary and medicinal herbs have indicated that they might be useful in preventing or treating cancer. A number of phytochemicals derived from these plants share common structures, as well as common properties as anti-inflammatory, anti-oxidant, anti-angiogenic and anti-cancer agents. Many studies have focused on traditional herbs used in Chinese or Ayurvedic medicine, but little research on herbal preparations used in traditional Native American medicine exists.

Goldenseal (Hydrastis canadensis L.) and bloodroot (Sanguinaria canadensis L) are two plants that have been used by Native Americans for medicinal purposes, including treatment of cancer. Goldenseal is a germicide and reduces inflammation. It was used by Native Americans, particularly the Cherokee Nation, as a breast cancer remedy. The dried root or rhizome of goldenseal was also used as a poultice to treat wounds and ulcers, and skin and eye ailments [1]. When mixed with bear’s grease, it was used as an insect repellent, diuretic and stimulant; the powder form was used in blister and cancer treatment [2]. The major alkaloid components of goldenseal are berberine (figure 1) and hydrastine with minor components of canadine and palmatine [3]. Berberine has been shown to inhibit HIV-1 reverse transcriptase [4]. Since many cancers can arise from DNA damage due to prior infection with retroviruses, berberine might have anti-cancer potential. Recent studies with animal models have demonstrated that bermurrubine, an analogue of berberine, functions as a topoisomerase poison and possesses anti-tumor activity [5]. It also induces apoptosis in promyelocytic leukemia HL-60 and 3T3 fibroblast cells and is effective in deactivation of carcinogens and tumor promotors [6].
Bloodroot (*Sanguinarine canadensis* L.) contains a benzophenanthridine alkaloid known as sanguinarine (figure 1) within its rhizomes. This phytochemical has broad antimicrobial and anti-inflammatory activities. Native American tribes such as the Cherokee, East Coast, and Lake Superior Indians utilized bloodroot extensively as a dye for body paint used in ritual ceremonies and in traditional medicine to treat sore throats, cough, rheumatoid arthritis and various cancers. This has been verified through documentation of indigenous medicinal plants during the 1800s, by the Lewis and Clark expedition. A syrup called “was-a-mos,” composed of roots of spiken, sweet fern, yellow dock, elecampane, vervain, pigeon cherry, white pine bark, and bloodroot was used by the Green Bay Indians to treat cancer [2]. Sanguinarine has been shown to inhibit NFkB, which plays an important role in the regulation of cell growth, cell cycle regulation and apoptosis [7]. A431 human squamous carcinoma cells treated with sanguinarine showed a decrease in viability, with higher doses triggering apoptosis. In K562 chronic myelogenous leukemia cells, 1.5 microg/ml induced apoptosis, while 12.5 microg/ml resulted in cell blister formation [8]. Sanguinarine has also been reported to induce apoptosis in multidrug resistant cervical carcinoma cells [9].

The purpose of this study was to examine in more detail the mechanisms by which berberine and sanguinarine inhibit cancer cell growth. Tests were conducted examining their effects on cell proliferation, DNA synthesis, and distribution of PCNA (proliferating cell nuclear antigen) and the cell cycle regulatory protein cyclin D1. Two relatively non-invasive (M10 and MCF-7) and two highly invasive (M2 and 231) melanoma and breast cancer cell lines were used in this study.

**Methods**

**Cell Culture Techniques**

M10 and M2 melanoma cells were a gift from Dr. Lillian Repesh (University of Minnesota, Duluth), and MCF-7 and 231 breast cancer cell lines were purchased from the American Type Tissue Culture Collection, (Manassas, VA). All cell lines were maintained in Dulbecco’s minimal essential media (DMEM) containing 7.5% fetal calf serum (Hyclone Inc., Logan, UT). Media was changed every 3-5 days, and cells were passaged and used for experiments by detachment with 0.05% trypsin and 0.5 mM EDTA in phosphate-buffered saline (PBS).
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**Proliferation Assay**

Cell proliferation was measured using the sulforhodamine B (SRB) assay as described by Skehan et al, 1990 [10]. MCF-7 cells were seeded at 5.0 x 10^4 cells/ml and M10 and M2 melanoma and 231 breast cancer cells were seeded at 1.0 x 10^4 cells/ml in 24-well plates and allowed to attach and recover for one or two days prior to drug treatment. Berberine and sanguinarine treated cultures received 6.25, 12.5, 25, 50 or 100 µM of phytochemical (Sigma Chemical Co., St. Louis, MO). Berberine was freshly prepared from a 100mM solution in DMSO and sanguinarine was prepared from a 50mM solution in dimethylsulfoxide (DMSO). Equal amounts of DMSO were added in vehicle control cultures. Control and phytochemical treated cells were cultured for four days without media change or drug replenishment. Media was then removed and the wells rinsed twice with PBS. Cells were fixed with cold 10% trichloroacetic acid on ice for 30 minutes, and the cells then rinsed twice with 70% ethanol and air dried. Cells were stained with 0.5% sulforhodamine B (Sigma Chemical Co., St. Louis, MO) for 30-60 minutes at room temperature, rinsed with 1% acetic acid to remove unbound dye, and air dried. The bound dye was solubilized with 10mM Tris-HCl buffer, pH 10, and absorbance was measured at 540 nm. For each experiment, all samples were run in duplicate or triplicate and the average was obtained. Experiments were repeated two to four times.

**Immunofluorescence Microscopy**

Each cell line was seeded on glass coverslips in six-well plates, as described in Section 2.2. After a one to two day recovery period, cells were treated with 100 µM (2 hrs) or 50µM (24 hrs) berberine or sanguinarine. Cells were fixed and stored in cold (-10 °C) absolute methanol. After fixation, the methanol was removed and cells were rinsed and rehydrated for 1 hour at 37 °C in 0.1% Tween-20 phosphate buffered saline (PBST) containing 1% carnation non-fat milk to prevent non-specific antibody labeling. Coverslips were incubated for 1 hour at 37 °C in antibodies to either PCNA or cyclin D1 (Santa Cruz Biotechnology, Inc, Santa Cruz, CA), washed three times in PBST, and then incubated in goat-anti-mouse-Texas red (Jackson ImmunoResearch, West Grove, PA) and 5µg/ml Hoechst 33258 (Sigma). Coverslips were mounted with anti-fade medium containing 90% glycerol, 10% PBS, and 0.1% para-phenylenediamine, and inspected and photographed on a Nikon Optiphot epifluorescence microscope using a Dage MTI SIT-68 video camera (Michigan City, IN).
To visualize DNA synthesis, cells treated with berberine or sanguinarine were pulse labeled for 30 minutes with 13 µM bromodeoxyuridine (BrdU) prior to fixation. These cells were methanol fixed as described above, and subsequently rehydrated with PBST and incubated in 2N HCl for 20 minutes at room temperature to denature the DNA. After several washes in PBST, cells were immunolabeled as described above using an anti-BrdU monoclonal antibody (Developmental Studies Hybridoma Bank, Iowa City, IA).

Results

Effects of Berberine and Sanguinarine on Melanoma and Breast Cancer Cell Proliferation

Dose response experiments show that the lowest concentrations of berberine and sanguinarine tested (12.5 µM) significantly inhibited the proliferation of all cell lines studied (table 1). Differences exist, however, in the sensitivity of the different cell lines to these phytochemicals. The ED$_{50}$ (effective dose at which all proliferation was 50% of control cultures) for berberine was 50 µM for the M10 and M2 melanoma cell lines; the ED$_{50}$ for sanguinarine was 25 µM for M10 cells and 12.5 µM for M2 melanoma, MCF-7, and 231 breast cancer cell lines. Combined treatment with both sanguinarine and berberine resulted in ED$_{50}$ values of 12.5 µM for 231 cells, and 25 µM for the M10 and M2 melanoma and MCF-7 breast cancer cell lines.

Effects of Berberine and Sanguinarine on DNA Synthesis in Melanoma and Breast Cancer Cell Lines

The effects of berberine and sanguinarine on BrdU (bromodeoxyuridine) incorporation differed among the cell lines tested. Neither berberine—nor sanguinarine—treated cells altered BrdU labeling patterns over the concentrations and time points examined (figure 4) in 231 cells. The other cell lines, however, displayed varying degrees of inhibition of BrdU labeling following drug treatment (figure 4). BrdU labeling was reduced in M2 and MCF-7 cells treated with 100 µM berberine for 2 hours; but not in cells treated with 50 µM of berberine for 24 hours. In contrast, sanguinarine completely blocked BrdU incorporation in M10 and MCF-7 cells at both short (100 µM for 2 hours) and long (50 µM for 24 hours) time points.
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Compared to controls, PCNA (proliferating cell nuclear antigen) labeling was not altered in 231 cells treated with berberine or sanguinarine over the concentrations and time points previously specified (figure 5). However, labeling intensity was reduced in berberine treated M10 and MCF-7 cells at short (100 µM, 2 hours), but not long (50 µM, 24 hours) time points (figure 5). PCNA labeling was reduced in sanguinarine treated M10 cells at both time points, and in M2 and MCF-7 cells treated with 50 µM sanguinarine for 24 hours and was completely suppressed in M2 and MCF-7 cells, exposed to 100 µM sanguinarine for 2 hours (figure 5).

Effects of Berberine and Sanguinarine on Cyclin D1 in Melanoma and Breast Cancer Cell Lines

Compared to control cells, cyclin D1 was not altered in 231 cells with either concentration or time point of berberine or sanguinarine (figure 6). Cyclin D1 was dimly labeled in M10 cells treated with berberine at both time points (figure 6). In contrast, M2 and MCF-7 cells treated with berberine were brightly labeled with cyclin D1 at both concentrations and time points (figure 6). In addition, cyclin D1 was distributed throughout nuclear space in these cell lines and there were fewer cells present with 100 µM of berberine for 2 hours (figure 6). Cyclin D1 was dimly labeled in M10 cells treated with 100 µM sanguinarine for 2 hours and there were fewer cells present in 50 µM sanguinarine treatment for 24 hours (figure 6). However, cyclin D1 was not labeled in M2 melanoma cells treated with 100 µM of sanguinarine for 2 hours, but were brightly labeled with 50 µM of sanguinarine for 24 hours, with distribution throughout nuclear space (figure 6). Cyclin D1 exited the nucleus and was completely redistributed into cytoplasmic space in MCF-7 cells treated with sanguinarine at both concentrations and time points (figure 6).

Effects of Berberine and Sanguinarine on Morphology of Melanoma and Breast Cancer Cell Lines

M2 melanoma and 231 breast cancer cells appeared highly apoptotic with 50 and 100 µM berberine and sanguinarine treatments, as indicated by cell shrinkage and blebbing (figures 4, 5, and 6). In addition, M2 melanoma cells appeared clumped with enlarged nuclei in both concentrations of berberine at either time point (figures 4, 5, 6). M10 melanoma cells, however, also appeared clumped with enlarged nuclei in 50 µM treatment of sanguinarine for 24 hrs. (figures 4, 5, 6).
Discussion

Berberine and sanguinarine have been previously reported to inhibit cell proliferation of different types of cancer cells including promyelocytic HL-60 and myelogenous leukemia cells, A431 human squamous carcinoma, multidrug resistant cervical cancer and MCF-7 breast cancer cells [6, 7, 8, 9]. Based on the cell proliferation assays and the overall appearance of treated cells viewed under an immunofluorescent microscope, this study found that sanguinarine is a more toxic phytochemical than berberine (figure 2).

The results of this study also indicate that 231 breast cancer cells were more sensitive to berberine than were M2 and M10 melanoma and MCF-7 breast cancer cells (table 1 and figure 3). In contrast, M2 melanoma cells were slightly more sensitive to sanguinarine than the other cell lines studied (table 1). 231 breast cancer cells were also more sensitive to co-treatment with berberine and sanguinarine; with the other cell lines exhibiting a slightly antagonistic response (figure 2). Interestingly, the breast cancer cell lines were more sensitive to either phytochemical than were the melanoma cell lines (table 1).

Some mechanisms by which berberine and sanguinarine can inhibit cell proliferation have been described. Studies have indicated that an analogue of berberine functions as a topoisomerase poison [5]. Sanguinarine has the ability to inhibit the action of the cell regulatory protein NFkB and induce blister formation and apoptosis in Bcl-2 expressing K562 myelogenous leukemia cells and multidrug resistant cervical carcinoma [7, 8, 9]. This experiment investigated the effects of these phytochemicals on DNA synthesis, visualized by the ability of berberine and sanguinarine to alter bromodeoxyuridine (BrdU) and proliferating cell nuclear antigen (PCNA) at a short time point (100 µM concentration for 2 hrs.) and a longer time point (50 µM concentration for 24 hrs.). In addition, effects on cyclin D1, the cell regulatory protein required for entrance into DNA synthesis phase, was investigated on these melanoma and breast cancer cell lines treated with the same concentrations of berberine and sanguinarine at the same time points.

Bromodeoxyuridine (BrdU) is a thymidine analog and is specifically incorporated into DNA during DNA synthesis [10]. BrdU is a useful tool in analyzing DNA synthesis, measured by 1) number of labeled cells, 2) fluorescence intensity, and 3) distribution within the nuclei. As with other types of labeling it is used in conjunction with Hoechst. Hoechst 33258, a bisbenzimide DNA intercalator used to stain nuclei, excites in the near UV (350 nm) region and emits in the blue region (450 nm) [11]. Thus,
the nuclei of cells present after drug treatment are stained blue, allowing for
the discernment of labeled versus non-labeled cells. These studies found
that BrdU labeling patterns were not drastically altered in either melanoma
or breast cancer cell line treated with berberine. However, labeling was
dimmer in these cell lines and there were fewer labeled cells (figure 4). In
contrast, sanguinarine was found to block BrdU incorporation in M10 and
MCF-7 cell lines at both concentrations and time points (figure 4). This is
consistent with the observation that sanguinarine is more toxic to these
cancer cell lines than berberine; it adds to the possibility that this may be
due to an inhibition or arrest of S-phase.

Proliferating cell nuclear antigen (PCNA) was also used to analyze
the effects of berberine and sanguinarine on DNA synthesis. PCNA is a
DNA polymerase delta accessory protein and is an indicator of the
proliferative state of of the cell. It is a repair substrate for ubiquitin and
assembles to a trimeric ring that encircles DNA and promotes replication
through DNA polymerase [12]. As in the BrdU experiments, effects on
PCNA were measured in conjunction with Hoechst-stained nuclei by
observing 1) the number of labeled nuclei, 2) the intensity of fluorescence,
and 3) the nuclear distribution. Results were that PCNA was not affected
in 231 cells treated with either berberine or sanguinarine (figure 5). However, PCNA was reduced in M10 and MCF-7 cell lines treated with
berberine at the short time point, but not at the longer time point (figure 5).
Compared to control cells, sanguinarine reduced PCNA labeling in M10
cells at both time points and in M2 and MCF-7 cells at the longer time
point (figure 5). PCNA was suppressed in M2 and MCF-7 cells at the
shorter time point (figure 5). Both concentrations and time points of
sanguinarine were able to inhibit DNA synthesis in M10 and MCF-7 cell
lines, yet PCNA was only slightly affected in M2 and MCF-7 cells at the
shorter time point; this suggests that there is not a relationship between the
effects of sanguinarine on DNA synthesis and alteration of PCNA in these
cell lines.

Cyclin D₁ is a cell regulatory protein found in G₁ phase. Its
presence in the nucleus is required for entry into S-phase. This is
accomplished by the binding of the retinoblastoma protein (pRb) to a
protein called E2F, which blocks transcription of S phase genes [13].
When cells are stimulated to divide by extracellular signals, active G₁-Cdk
accumulates and phosphorylates pRb, reducing its affinity for E2F [13].
The retinoblastoma protein then dissociates, allowing E2F to activate S-
phase gene expression [13]. This study found that neither berberine nor
sanguinarine had an effect on cyclin D₁ in 231 breast cancer cells (figure 6).
Cyclin D₁ was dimly labeled in M10 cells treated with berberine at both
concentrations and time points (figure 6). In contrast, M2 and MCF-7 cells treated with berberine were brightly labeled with cyclin D1. Cyclin D1 labeling was dimmer than the controls in M10 cells treated with sanguinarine for the short time point, with fewer cells present in sanguinarine treatment at the longer time point (figure 6). M2 cells treated with sanguinarine at the short time point were not labeled with cyclin D1 and brighter labeling was observed in the longer time point with nuclear expansion (figure 6). The most striking effect was seen in MCF-7 cells treated with sanguinarine. Cyclin D1 was completely absent from the nuclei, but present in the cytoplasm in both the early and long time points (figure 6). These results indicate that berberine and sanguinarine did not have a major effect on cyclin D1 distribution in either melanoma cell line or 231 breast cancer cell line. But interestingly, sanguinarine created a drastic redistribution of cyclin D1 from nucleus to cytoplasm in the MCF-7 breast cancer cell line. This could account for the total inhibition of BrdU uptake in MCF-7 cells treated with both concentrations and time points of sanguinarine.

Immunofluorescence microscopy experiments revealed that M2 melanoma and 231 breast cancer cells treated with either berberine or sanguinarine at either concentration and time point appeared highly apoptotic, as characterized by cell shrinkage, clumping, and blebbing. The mechanism by which apoptosis occurs in these cell lines with phytochemical treatment should be studied. A number of mechanisms that induce apoptosis include direct caspase activation or indirect caspase activation, such as through c-myc, Apaf-1 or Bcl-2 expression [14, 15, 16].

In summary, despite having similar chemical structures, berberine and sanguinarine have different toxicities and effects on these melanoma and breast cancer cell lines. These differences do not appear to be related to invasiveness, but rather vary among each cell type. For example, the more highly invasive breast cancer cell line (231) was more sensitive to the less toxic phytochemical, berberine (figure 3). In addition, immunofluorescence microscopy experiments revealed that 231 cells treated with berberine appeared highly apoptotic. Perhaps berberine acts on 231 breast cancer cells by directly triggering a caspase cascade or triggering molecules responsible for caspase activation, such as INF gamma, c-myc, or Bcl-2 [14, 15, 16]. Or perhaps 231 cells are particularly sensitive to berberine due to an effect on pS2 and capthsin D promoters related to the invasiveness associated specifically with 231 breast cancer cells [17]. Future experimentation with berberine should explore some of these apoptosis-inducing mechanisms in 231 breast cancer cells, as well as explore the genesis of the time at which breast cancer cells become invasive.
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to form the 231 phenotype and the ways in which berberine might be affecting this. In contrast, the more invasive melanoma cell line (M2) was slightly more sensitive to the more toxic phytochemical, sanguinarine (table 1). Immunofluorescence experiments showed that BrdU was inhibited in M2 melanoma cells treated with sanguinarine, but not with PCNA or cyclin D1 (figure 4). This could mean that sanguinarine has the ability to arrest the cell cycle in M2 melanoma by mechanisms other than those explored in this study, such as by other cyclin-dependent kinases or proteins such as p53 [13]. This study also found that sanguinarine has the ability to cause redistribution of cyclin D1 from the nucleus to the cytoplasm in the less invasive, estrogen receptive, MCF-7 breast cancer cells (figure 6). This could explain the lack of BrdU labeling in sanguinarine treated MCF-7 cells. However, PCNA was not affected in MCF-7 cells treated with sanguinarine (figure 5), suggesting that if a cell cycle arrest occurred, it did not affect all cells equally. Lastly, this study reported morphological changes observed in M2 melanoma and 231 breast cancer cells with berberine and sanguinarine treatment consistent with cells undergoing apoptosis. Thus, any dim labeling of PCNA or cyclin D1 observed in these cell lines, could be a result of nonspecific binding of remnant protein.

Future research should explore these two phytochemicals on other cancer cell lines to see if effects on proliferation and DNA synthesis are similar or completely novel. Because micromolar concentrations of these phytochemicals had a significant effect on cell proliferation, there exists the possibility for future applications on higher organisms. Thus, experiments treating non-cancerous cells with berberine and sanguinarine should be done. Another experiment should be conducted to decipher why the more invasive breast cancer cell line (231) was more sensitive to the less toxic phytochemical (berberine). Cyclin D1 had a dramatic redistribution from nucleus to cytoplasm in sanguinarine treated MCF-7 cells. Studies should be conducted to verify the substituent of sanguinarine responsible for this redistribution.

Perhaps the various effects on cell proliferation and the cell cycle with berberine and sanguinarine treatment on these melanoma and breast cancer cell lines might explain why goldenseal and bloodroot were used in traditional Native American medicine and why they might have been potent anti-cancer agents. Therefore more research attention should be paid towards traditional Native American plants as possible chemotherapeutics and novel mechanistic approaches to biomedical research.
Acknowledgements

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Table 1: Effective Dose at 50% cell growth reduction for all cell lines with sanguinarine and berberine (ED$_{50}$) and dose at which there is a significant difference from the control (SD).

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Figure 1: Chemical structures of (a.) berberine and (b) sanguinarine. (Notice the similarity among their structures with the cyclopentene and cyclic aromatic rings.)
Figure 2: SRB cell proliferation assay of, (a) M10 and (b) M2 melanoma and (c) MCF-7 and (d) 231 breast cancer cells with 4 day treatment with 6.25 µM, 12.5 µM, 25 µM, 50 µM and 100 µM berberine and/or sanguinarine. Overall sanguinarine was more effective than berberine at reducing cell growth and combination of berberine and sanguinarine had, if any, a slightly antagonistic effects. Error bars represent p-value < 0.5.

Figure 3: SRB cell proliferation assay comparing the effect of (a) berberine and (b) sanguinarine against both melanoma and breast cancer lines studied. Sanguinarine was more toxic to cells than berberine. While all cell lines responded approximately equal to sanguinarine, 231 breast cancer cells were more sensitive to berberine. Error bars represent p-value < 0.5.
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![Image showing the effects of different concentrations of alkaloids on M10 and M2 melanoma cell lines.](image)

a.
b.

**Figure 4:** Comparison of the effects of berberine and sanguinarine on DNA synthesis in a) M10 and M2 melanoma and b) MCF-7 and 231 breast cancer cells. Hoechst stains nuclei and BrdU labeling measures the number of cells in S-phase. (Note total inhibition of BrdU in sanguinarine treated M10 and M2 melanoma and MCF-7 breast cancer cell lines.)
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<table>
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a.
Figure 5: Comparison of the effects of berberine and sanguinarine on DNA synthesis/polymerase in a) M10 and M2 melanoma and b) MCF-7 and 231 breast cancer cells. Hoechst stains nuclei and PCNA measures direct effects on DNA synthesis through polymerase binding. (Note that in comparison to the controls there are no major changes in PCNA distribution in either melanoma or breast cancer cell line and sanguinarine treated M10 and M2 melanoma and 231 breast cancer cell lines appear highly apoptotic.)
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![Cell Images](m10_melanoma_hoechst_cyclin_d1.png)  ![Cell Images](m2_melanoma_hoechst_cyclin_d1.png)

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Figure 6: Comparison of the effects of berberine and sanguinarine on cyclin D₁ distribution in a) M10 and M2 melanoma and b) MCF-7 and 231 breast cancer cells. Cyclin D₁ is required for entrance into S-phase. Note the striking redistribution of cyclin D₁ out of the nucleus and into the cytoplasm in MCF-7 breast cancer cells. M2 melanoma and 231 breast cancer cells appear highly apoptotic in berberine or sanguinarine treatment.
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