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# Homeopathic Medicines Do Not Alter Growth and Gene Expression in Prostate and Breast Cancer Cells In Vitro

Rajesh L. Thangapazham, MS, Jaya P. Gaddipati, PhD, N. V. Rajeshkumar, PhD, Anuj Sharma, MS, Anoop K. Singh, PhD, John A. Ives, PhD, Radha K. Maheshwari, PhD, and Wayne B. Jonas, MD

**Background:** Homeopathy is an alternative medical system practiced in all parts of the world. Although several theories are proposed to explain the mechanisms of action, none are scientifically verified. In this study, the authors investigate the effect of selected homeopathic remedies often used to treat prostate and breast cancer. **Materials and Methods:** The authors investigated the effect of the homeopathic medicines *Conium maculatum*, *Sabal serrulata*, *Thuja occidentalis*, *Asterias*, *Phytolacca*, and *Carcinosin* on prostate and breast cancer cell (DU-145, LNCaP, MAT-LyLu, MDA-MB-231) growth and on gene expression that regulates apoptosis, using MTT and multiprobe ribonuclease protection assay. **Results:** None of the homeopathic remedies tested in different potencies produced significant inhibitory or growth-promoting activity in either prostate or breast cancer cells. Also, gene expression studies by ribonuclease protection assay produced no significant changes in mRNA levels of bax, bcl-2, bcl-x, caspase-1, caspase-2, caspase-3, Fas, or FasL after treatment with homeopathic medicines. **Conclusions:** The results demonstrate that the highly diluted homeopathic remedies used by homeopathic practitioners for cancer show no measurable effects on cell growth or gene expression in vitro using currently available methodologies.

**Keywords:** homeopathy; DU-145; LNCaP; MAT-LyLu; MDA-MB-231; apoptosis; carnosin

Homeopathy is a system of medicine developed in the late 18th century by Samuel Hahnemann based on the principle “like cures like” and often uses extremely high dilutions of remedies. Most, but not all, critical reviews of clinical literature in homeopathy demonstrate significant effects beyond placebo, but there is an insufficient research base in any one condition to demonstrate specific efficacy or to understand mechanisms.<sup>1,2</sup> The literature includes only a few high-quality studies on the use of homeopathy in cancer.<sup>3</sup> Despite more than 200 controlled clinical trials on homeopathy, the heterogeneity and insensitiv-

ity of clinical research models has led to an impasse in further understanding of this system of medicine in any one biological or clinical area.<sup>4</sup> Well-organized studies with sound methodological analysis are lacking to date, and better research is needed.<sup>5</sup> The skepticism and controversy surrounding clinical observations of beneficial effects from homeopathic treatments may account for the few scientific investigations into possible mechanisms for the professed therapeutic effects of high-dilution remedies. In a recent study, we examined the effect of prostate cancer MAT-LyLu cell-injected Copenhagen rats given homeopathic treatment containing *Thuja occidentalis*, *Conium maculatum*, *Sabal serrulata*, and MAT-LyLu cell *Carcinosin*. There was significant reduction in the tumor incidence (23%), tumor volume (45%) and tumor weight (33%) in the homeopathy-treated group as compared with the control group.<sup>6</sup> Blind comparison of visible lung metastases and histopathological examination demonstrated a few small nodules in the homeopathy-treated tumor-bearing animals compared to the untreated group having numerous unencapsulated nodular accumulations of large size. Homeopaths have proposed systemic memory hypotheses<sup>7-9</sup> to account for the therapeutic effects of homeopathic medicines. Lattice formations created by water molecules and other biophysical mechanisms during the serial dilutions have also been proposed as mechanisms, but all these lack scientific proof.

Because of this, we are interested in investigating the mechanisms underlying homeopathic treatments. In this study, we investigated the effect of homeopathic medicines in vitro on cell growth and gene expression.

RLT, JPG, NVR, AS, AKS, and RKM are in the Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland. RLT and AS are also at the Birla Institute of Technology and Science, Pilani, India. JAI and WBJ are at the Samuelli Institute, Alexandria, Virginia.

**Correspondence:** Wayne B. Jonas, MD, Samuelli Institute, 1700 Diagonal Road, Suite 400, Alexandria, VA 22314. E-mail: wjonas@siib.org.

We examined the effect of *C maculatum*, *S serrulata*, *Thuja occidentalis*, and MAT-LyLu *Carcinosin* on prostate cancer cells (DU-145 and LNCaP) and *C maculatum*, *Asterias*, *Phytolacca*, and MDA-MB-231 *Carcinosin* on breast cancer cells (MDA-MB-231). Our previous studies by immunohistological analysis of tumor tissues have indicated induction of apoptosis in tissues from homeopathy-treated animals.<sup>10</sup> In this article, we examine the underlying changes in the expression of apoptotic genes in prostate and breast cancer cell models.

## Materials and Methods

### Homeopathic Medicines

Homeopathic medicines *C maculatum*, *S serrulata*, *T occidentalis*, *Asterias*, and *Phytolacca* with 30 c, 200 c, and 1000 c concentrations were obtained from Boiron (Simi Valley, Calif). *Carcinosins* (1000 c) were prepared from MAT-LyLu and MDA-MB-231 cells by Washington Homeopathic Products Inc (Bethesda, Md), as previously described.<sup>10</sup> Briefly, for *Carcinosin* preparation, MAT-LyLu cells grown to 80% confluence were trypsinized and washed twice with cold phosphate-buffered saline. The cells were resuspended in 75% ethanol and passed through a 26-gauge syringe needle 10 to 15 times. A 0.1-mL suspension was diluted with 9.9 mL of 75% ethanol. Subsequent dilutions to 1000 c were done in water by Washington Homeopathic Products Inc. Controls were diluted and shaken in tap water.

### Cells and Treatment

Human prostate cancer cells DU-145 and LNCaP, rat prostate cancer cells MAT-LyLu, and human breast cancer cells MDA-MB-231 were obtained from American Type Culture Collection (ATCC; Manassas, Va). Cells were maintained as monolayer cultures in media as recommended by ATCC in a humidified incubator containing 5% CO<sub>2</sub> at 37°C.

### Cell Growth and Viability

Prostate cancer cells MAT-LyLu, DU-145, and LNCaP and breast cancer cells MDA-MB-231 were seeded at 1.5 to 2 × 10<sup>4</sup> cells per well in 96-well plates. Homeopathic medicines were applied in different sets of plates by adding 100 µl 30 c, 200 c, and 1000 c concentrations for *T occidentalis*, *C maculatum*, and *S serrulata* and 1000 c for MAT-LyLu *Carcinosin* for prostate cells and *C maculatum* (1000 c), *Asterias* (30 c), and *Phytolacca* (30 c) and MDA-MB-231 *Carcinosin* (1000 c) for breast cancer cells. Cells in untreated wells served as controls. Cells were allowed to grow for 24, 48, 72, and 96 hours. The respective effect on cell growth and viability in the presence of various doses of homeopathic drugs was determined using the Cell

Proliferation Kit I (MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) obtained from Roche Diagnostics GmbH (Germany). After completing treatment, cells were incubated with MTT for 3 to 4 hours at 37°C. Cells were lysed, and then the reduced intracellular formazan product was dissolved in the solubilization buffer provided in the kit. MTT is reduced to a colored water-insoluble formazan salt only by metabolically active cells, which is quantitated in a conventional enzyme-linked immunosorbent assay (ELISA) plate reader at 570 nm.

### mRNA Analysis by Ribonuclease Protection Assay

To determine the effect of homeopathic drugs on mRNA expression, MAT-LyLu cells were cultured in 75-cm<sup>2</sup> flasks and were treated with *C maculatum* and *S serrulata* at concentrations of 0 to 1000 c for 48 hours. Following the treatments, cells were harvested, and total RNA was prepared using the Trizol method (Life Technologies, Gaithersburg, Md). The isolated RNA was quantitated by spectrophotometry and equalized, and the purity was checked on 1% formaldehyde agarose gel. mRNA expression of treated and untreated cells was determined by ribonuclease protection assay (RPA). mRNA levels for apoptotic genes were estimated using RiboQuant multiprobe set rAPO-1 (bax, bcl-2, bcl-x, caspase-1, caspase-2, caspase-3, Fas, FasL, L32, and GAPDH) using a kit from BD Biosciences (San Diego, Calif). The protocols used for the RPA were according to the manufacturer's instructions. Briefly, 20 µg of each RNA sample was hybridized at 56°C for 12 to 14 hours with a <sup>32</sup>P-UTP-labeled probe. The probe was prepared by transcribing the rat apoptosis template set using T7 RNA polymerase. After hybridization, samples were subjected to RNase digestion for 45 minutes at 30°C. The ribonuclease-protected bands were then resolved on denaturing urea-polyacrylamide gels, followed by autoradiography. L32 and glyceraldehyde 3-phosphate dehydrogenase mRNAs served as housekeeping gene controls in the assay to ensure equal loading of RNAs.

## Results

### Effect of Homeopathic Medicine on Cell Growth and Viability

Homeopathic medicines showed no significant effect on the growth and viability in all 3 cell lines (Figures 1-3). The effect was not significant at any of the concentrations or time points. Similarly, MDA-MD-231 breast cancer cells showed no changes in their growth pattern (Figure 4). No morphological alterations or other changes were observed with any of the cells and treatments.

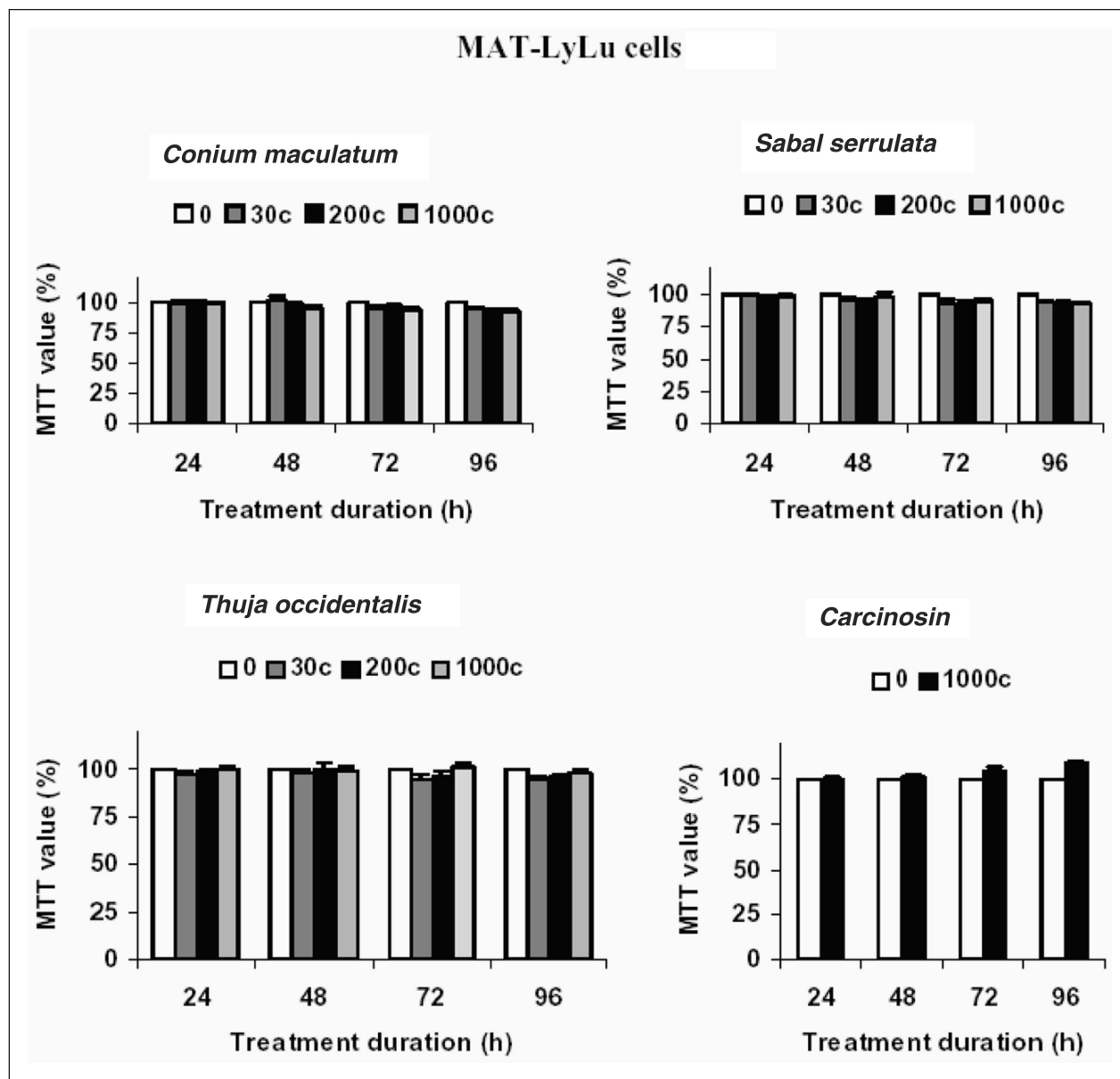


Figure 1 Effect of homeopathic medicines (*Conium maculatum*, *Sabal serrulata*, and *Thuja occidentalis* at concentrations of 30 c, 200 c, and 1000 c and Carcinosisin at 1000c) on the growth of MAT-LyLu cells treated for different durations (24, 48, 72, and 96 hours). MTT values are expressed in percentage compared to control and are mean + SE from 6 observations.

### Effect of Homeopathic Medicines on Apoptotic Gene Expression

To determine whether there is a modulation in the mRNA expression of genes that regulate apoptosis (bax, bcl-2, bcl-x, caspase-1, caspase-2, caspase-3, Fas, FasL) from homeopathic medicine, RPA analysis of total RNA was performed (Figure 5). After normalization of densitometric estimations with that of house-keeping genes L32 and GAPDH, the data showed that treatment of MAT-LyLu cells with different concentrations of *C maculatum* or *S serrulata* produced no significant changes in their expression compared to untreated control cells.

### Discussion

Homeopathy administers minute quantities of substances that produce symptoms of illness in a healthy person when administered in large doses. However, there are few studies available examining the mechanisms of homeopathy using conventional scientific methods. In the present study, we examined the effect of these medicines at the cellular level using prostate and breast cancer cells.

According to the homeopathic system of medicine, remedies in high serial dilutions retain their biological activity,<sup>3</sup> have reduced toxicity, and invoke healing processes. In a previous study, we found that selected

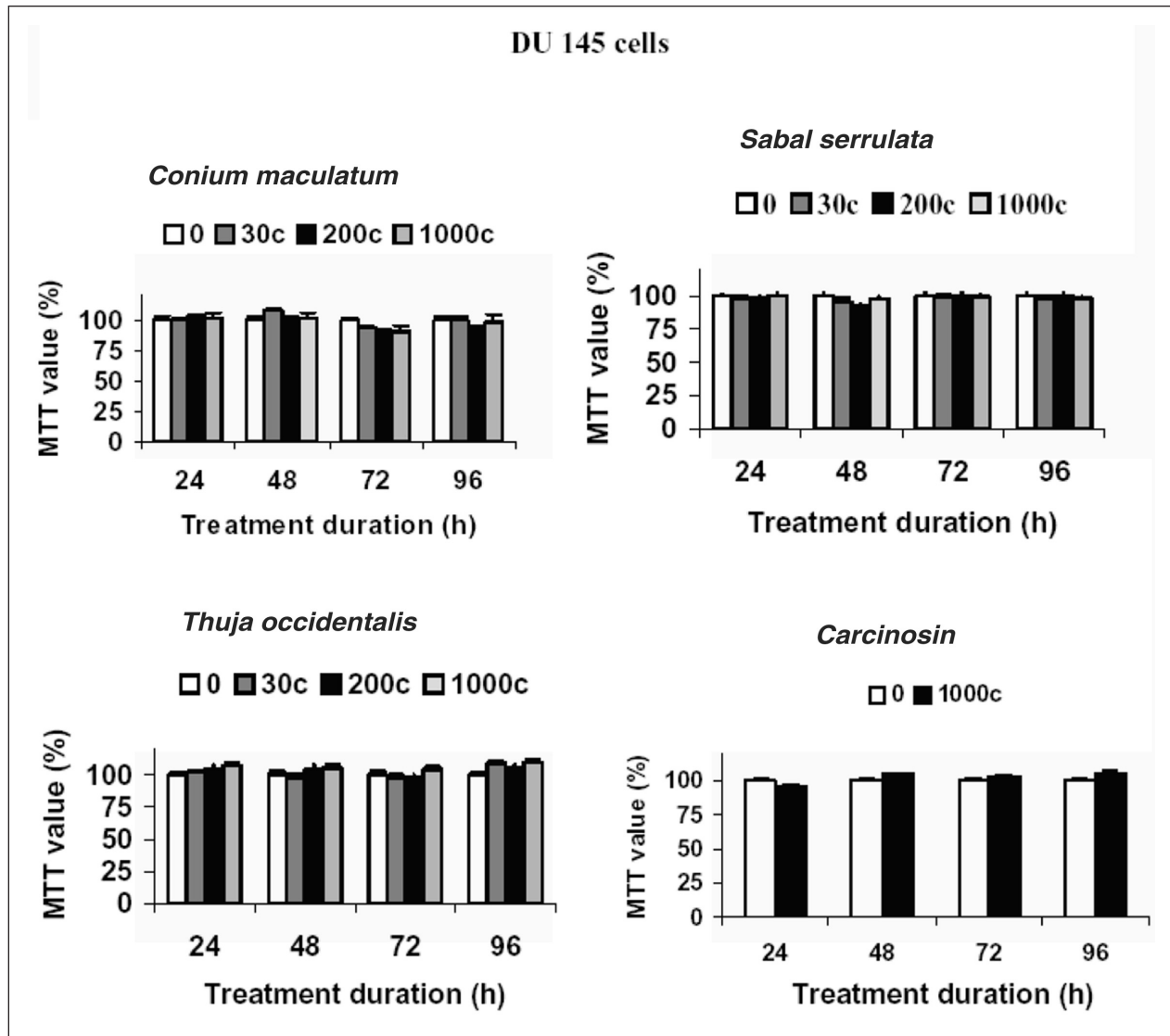


Figure 2 Effect of homeopathic medicines (*Conium maculatum*, *Sabal serrulata*, and *Thuja occidentalis* at concentrations of 30 c, 200 c, and 1000 c and *Carcinosin* at 1000 c) on the growth of DU-145 cells treated for different durations (24, 48, 72, and 96 hours). MTT values are expressed in percentage compared to control and are mean + SE from 6 observations.

remedies commonly used in the clinical treatment of prostate cancer reduced cancer progression in an animal model.<sup>6</sup> In this study, we found no significant difference between the homeopathy-treated subjects and controls and on cell proliferation or inhibiting growth. These results indicate that they are not cytotoxic, a finding also reported by others.<sup>11</sup> This supports the notion that homeopathic medicines in high dilutions are safe but does not support the claims that they are effective against cancer.<sup>10,12-14</sup>

Apoptosis, or programmed cell death, is a mechanism cells adopt to remove abnormal cells, and it plays a vital role in development and in many other biological functions. Apoptosis plays a pivotal role in the control of tumor growth by counterbalancing proliferation.<sup>15</sup> In a preliminary study of homeopathic

drugs against tumor growth and development in a prostate cancer model in the Copenhagen rat, we observed reduced tumor incidence and tumor burden in the homeopathic treatment group.<sup>6</sup> Tumor tissue analysis indicated a reduction in proliferation and also an increase in apoptotic cells in tissues from homeopathically treated specimens compared to water-treated controls. Earlier reports have shown that low doses of toxic substances can enhance the mRNA levels of heat shock protein hsp70<sup>16</sup> in mammalian cells. The homeopathic drug *Arsenicum Album* at low doses was shown to alter protein and biochemical profiles in mice.<sup>17</sup> In our laboratory, we have shown up-regulation of metallothionein protein in human cells exposed to low doses of cadmium for longer durations.<sup>18</sup> However, in this study, cells

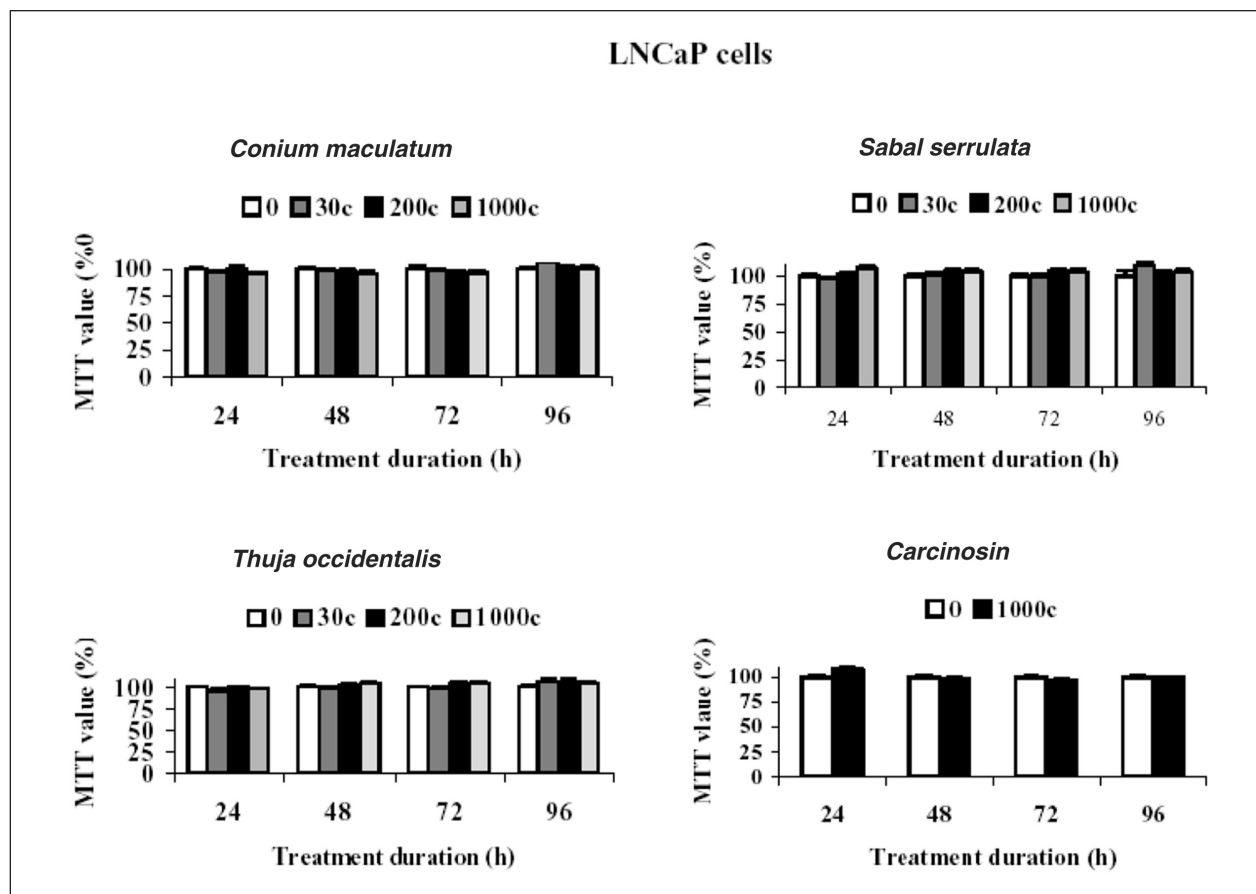


Figure 3 Effect of homeopathic medicines (*Conium maculatum*, *Sabal serrulata*, and *Thuja occidentalis* at concentrations of 30 c, 200 c, and 1000 c and *Carcinosin* at 1000 c) on the growth of LNCaP cells treated for different durations (24, 48, 72, and 96 hours). MTT values are expressed in percentage compared to control and are mean + SE from 6 observations.

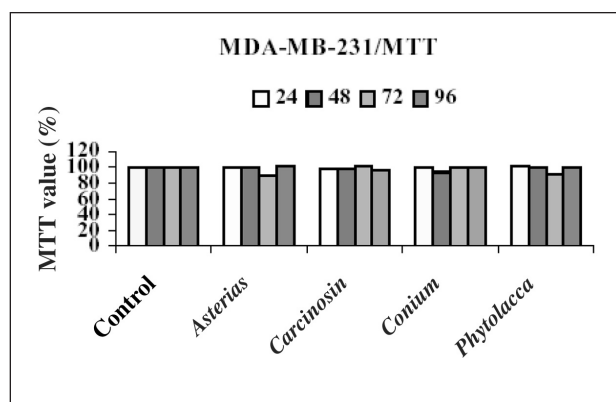


Figure 4 Effect of homeopathic medicines (*Conium maculatum* [1000 c], *Asterias* [30 c], *Phytolacca* [30 c], and *Carcinosin* [1000 c]) on the growth of MDA-MB-231 cells treated for different durations (24, 48, 72, and 96 hours). MTT values are expressed in percentage compared to control and are mean + SE from 6 observations.

treated with low-dose homeopathic drugs for short durations did not cause any significant changes in the apoptotic genes analyzed. Studies using in vivo models with intact immune mechanisms may be more

appropriate than cell models to study these low-dose treatments.

## Conclusions

In conclusion, we found no evidence that homeopathic remedies previously shown to inhibit cancer in animal models were proliferative or cytotoxic when studied in isolated cell models. If homeopathy works, its mechanisms may be complex, requiring both in vivo and in vitro screening and verification. Whole genome arrays exploring complex adaptogenic patterns may also be needed to understand its subtle and often elusive effects.

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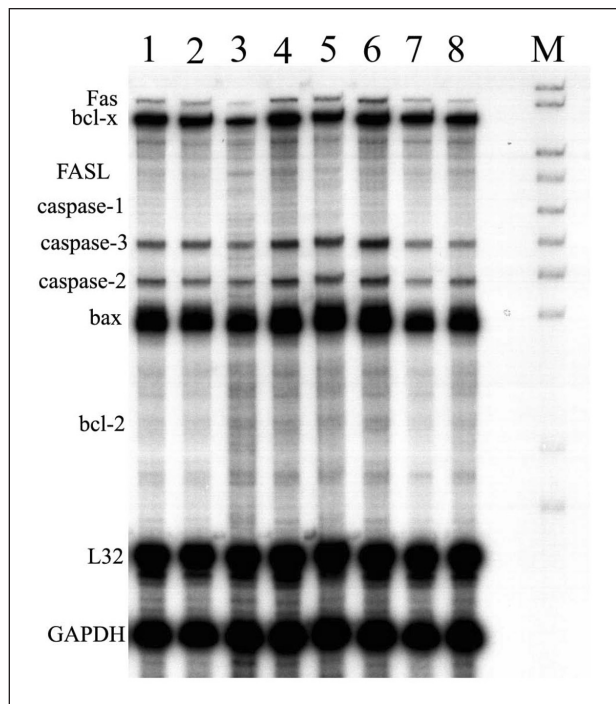


Figure 5 mRNA expression of apoptotic genes in MAT-LyLu cells as analyzed by ribonuclease protection assay. Lanes: M = marker; MAT-LyLu cells treated with *Conium maculatum* (lane 1, 0 c; lane 2, 30 c; lane 3, 200 c; and lane 4, 1000 c), and *Sabal serrulata* (lane 5, 0 c; lane 6, 30 c; lane 7, 200 c; and lane 8, 1000 c).

and should not be construed as official or necessarily reflecting the views of the USUHS or the Department of Defense. The authors are thankful for the excellent technical assistance of Brant Freed and manuscript preparation by Cindy C Crawford.

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