Effects of Homeopathic Preparations on Human Prostate Cancer Growth in Cellular and Animal Models

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The use of dietary supplements for various ailments enjoys unprecedented popularity. As part of this trend, *Sabal serrulata* (saw palmetto) constitutes the complementary treatment of choice with regard to prostate health. In homeopathy, *Sabal serrulata* is commonly prescribed for prostate problems ranging from benign prostatic hyperplasia to prostate cancer. The authors' work assessed the antiproliferative effects of homeopathic preparations of *Sabal serrulata*, *Thuja occidentalis*, and *Conium maculatum*, in vivo, on nude mouse xenografts, and in vitro, on PC-3 and DU-145 human prostate cancer as well as MDA-MB-231 human breast cancer cell lines. Treatment with *Sabal serrulata* in vitro resulted in a 33% decrease of PC-3 cell proliferation at 72 hours and a 23% reduction of DU-145 cell proliferation at 24 hours ($P < .01$). The difference in reduction is likely due to the specific doubling time of each cell line. No effect was observed on MDA-MB-231 human breast cancer cells. *Thuja occidentalis* and *Conium maculatum* did not have any effect on human prostate cancer cell proliferation. In vivo, prostate tumor xenograft size was significantly reduced in *Sabal serrulata*–treated mice compared to untreated controls ($P = .012$). No effect was observed on breast tumor growth. Our study clearly demonstrates a biologic response to homeopathic treatment as manifested by cell proliferation and tumor growth. This biologic effect was (i) significantly stronger to *Sabal serrulata* than to controls and (ii) specific to human prostate cancer. *Sabal serrulata* should thus be further investigated as a specific homeopathic remedy for prostate pathology.

Keywords: *prostate cancer; Sabal serrulata; Thuja occidentalis; Conium maculatum; human prostate and breast cancer cell lines; nude mice*

Prostate cancer remains the second leading cause of cancer-related deaths in men in the United States, with an estimated 30 350 deaths and 232 090 new cases in 2005 alone.[1] The worldwide incidence of prostate cancer deaths has been estimated at 204 000 per year.[2] In the European Union, approximately 100 000 new diagnoses and 40 000 deaths are reported annually.[3] Various molecular mechanisms are involved in the pathogenesis, proliferation, and metastasis of prostate cancer, and significant controversy surrounds the management of this major public health problem. Although detection of the disease is possible at an early stage using either serum prostate specific antigen measurements or biopsies, screening has not been shown to improve survival.[4] Conventional treatments for early-stage disease involve watchful waiting, radiotherapy, and prostatectomy, depending on patient age and comorbidities. In the case of metastatic prostate cancer, androgen suppression remains the treatment of choice after more than 50 years. Orchiectomy or hormonal therapy, such as LH-RH agonists and antiandrogens, is used as systemic therapy designed to prevent access of androgens to the prostate cancer cells. The major complications of this therapeutic approach are osteoporosis and osteoporosis-related fractures, with approximately 2000 cases per year in the United States alone.[5] Many factors, such as peptide and steroid hormones, growth factors, and cytokines, appear to be involved in the pathogenesis, the aggressiveness, and the metastatic potential of prostate cancer.[6] Scientists are investigating possible causes at the dietary, genetic, and molecular levels in an attempt to identify new avenues to prevent, detect, diagnose, and treat this disease.[7]

Phytotherapy has long constituted an important component of medical treatment in most societies. The pharmaceutical industry used medicinal herbs

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to isolate and characterize active ingredients, relegating the natural compounds to prototypes for a variety of synthetic molecules designed for enhanced specificity and efficacy. About 25% of prescription drugs contain at least one active ingredient derived from plant material.[8] Unfortunately, in many cases the dream of a "miracle drug" did not materialize and left health care providers struggling with adverse effects and toxicities. As a consequence, the medical, pharmaceutical, and scientific communities are reconsidering the merits of herbal therapies, which have continued to play a significant role in many parts of the world. Medicinal herbs are used either in toto or as parts of a plant (eg, roots, flowers, fruit) in a decoction, infusion, or cataplasm form, as is the case in traditional Chinese medicine, Greco-Unani medicine, or homeopathy. The latter is among the most widely accepted complementary and alternative medicine (CAM) modalities in Western Europe. A survey assessing the extent of CAM teaching at European Union Universities revealed that the topic of homeopathy is the number 1 subject, followed by acupuncture and phytotherapy.[9] In India and Latin America, homeopathy remains a widespread and popular form of medical treatment. In contrast, homeopathy in the United States continues to lag behind other CAM modalities, which are considered less controversial. "Similia similibus curentur" or treating "like with like" is the central therapeutic principle in homeopathy. It means that a patient favorably responds to a particular homeopathic remedy capable of eliciting his or her symptoms in a healthy individual. These homeopathic medicines are administered in very highly diluted preparations. As these "ultra-molecular" dilutions seemingly defy the principles of pharmacology,[10] the allopathic medical community remains skeptical and demands scientific evidence supporting their use.

With regard to homeopathic scientific evidence, laboratory research has focused on the cellular immune response to inflammation and on toxicology-based in vivo models. A series of experiments conducted by French scientists demonstrated the degranulation of human basophils by a very dilute anti-IgE antiserum.[11] The study led to a wave of criticism and triggered significant controversy in the scientific community, as other researchers were unable to duplicate the group's findings.[12] However, recent investigations confirm that basophil degranulation is indeed modulated by homeopathic histamine administration.[13,14] With regard to the above-mentioned toxicology-based approach, a number of research models have managed to demonstrate a homeopathic therapeutic effect. Heavy metal toxicity induced in laboratory animals was alleviated with homeopathic dilutions of arsenic and bismuth (7c) via an increase in urinary heavy metal elimination.[15,16] Bildet and colleagues showed a protective effect of carbon tetrachloride (7c) and phosphorus (7c and 15c) on carbon tetrachloride-induced hepatitis in rats.[17,18] Similarly, mortality of mice due to mercury-induced nephrotoxicity was reduced when treating with 9c and 15c *Mercurius corrosivus*.[19] Yet an additional experimental setting successfully demonstrated the protective and curative properties of Apis (7c and 9c) on x-ray-induced erythema in albino guinea pigs.[20,21] Homeopathic dilutions of cadmium and cisplatin applied to cultured kidney cells confer protection against these same substances when administered in pharmacological concentrations.[22] Furthermore, neuroprotective effects of ultra-low-dose glutamate in the context of glutamate-induced primary rat spinal, cortical, and cerebellar neuronal toxicity have also been reported.[23]

In the field of cancer research, a very limited number of reports addressing homeopathy are found in the scientific literature. *Ruta graveolens* selectively induces cell death in brain cancer cells while promoting proliferation in normal peripheral blood lymphocytes.[24] Amelioration of *p*-dimethylaminoazobenzene-induced hepatocarcinogenesis in mice treated with homeopathic *Chelidonium* either alone[25] or in combination with carcinosin was reported.[26] A complex homeopathic remedy frequently prescribed by Brazilian physicians for immunodeficiency disorders was tested on sarcoma 180-bearing mice. Significant tumor regression was noted in the treatment group, indicating the remedy's anticancer effect.[27] In spite of the disease's increasing significance, no in vitro or in vivo studies on the use of homeopathy for prostate cancer have been reported.

Anecdotal clinical evidence collected from homeopathic treatment centers supports the beneficial effects of homeopathy in alleviating and curing prostate problems, including cancer. The most commonly prescribed remedy for prostate pathology is *Sabal serrulata* (synonym to *Serenoa repens* [W. Bartram] Small, family Arecaceae, commonly known as saw palmetto). Depending on the patient's individual symptomatology, other remedies, such as *Thuja occidentalis*, *Conium maculatum*, and carcinosin, are at times added to the regimen. In Western Europe, *Sabal serrulata* is a popular phytotherapeutic agent for the treatment of uncomplicated prostatism of middle and later life. The favorable European experience has been the major impetus for the current interest in the United States. Despite some limitations, there is increasing evidence that herbal *Sabal serrulata* extract exerts beneficial effects by improving a number of urological symptoms and flow measures.[28,29] Urologists thus increasingly consider *Sabal serrulata* extract for men with symptomatic benign prostate hypertrophy (BPH). In a recently published review of clinical trials, Gerber and Fitzpatrick reported the significant effect of *Sabal serrulata* in reducing the symptoms of BPH,

increasing urinary flow, and improving the quality of life. They concluded that *Sabal serrulata* extract might well be considered a viable first-line therapeutic modality in the treatment of prostate disorders.[30] However, a more recent randomized, double-blind clinical trial investigating the effects of *Sabal serrulata* showed no benefits over placebo and no improvement of BPH symptoms.[31] Thus, reports about its efficacy remain controversial. Although frequently prescribed by homeopaths, no studies or published research on *Thuja occidentalis*, *Conium maculatum*, or carcinosin pertaining to prostate pathology are available.

In this study, we used a series of homeopathic preparations commonly prescribed to patients suffering from prostate pathology ranging from benign prostatic hyperplasia to prostate cancer. We therefore sought to investigate the effects of these above-mentioned homeopathic preparations on the proliferation of human prostate cancer cell lines in vitro and on tumor growth in nude mice bearing prostate tumors and breast tumors as control.

Materials and Methods

Homeopathic Treatments

Mother tinctures and homeopathic preparations of *Sabal serrulata*, *Thuja occidentalis*, *Conium maculatum*, and carcinosin, as well as homeopathically succussed deionized water, were prepared by Hyland's (Paoli, PA). Mother tinctures were stored in 67% alcohol, and homeopathic solutions were prepared at minus 5 of final potentization and stored in 87% alcohol. All potentizations up to 200 CH were performed in Hahnemannian method (Hahneman used a fresh vial for each step of the potentization) and Korsakovian method beyond 200 (300-1000 CK) (Korsakoff used the same vial that he emptied between each potentization step). Only the final 5 potentizations of homeopathic treatments were performed in our laboratory.

Cell Cultures

The androgen receptor negative human prostate cancers DU-145 and PC-3, and human breast cancer MDA-MB-231 cell lines were obtained from the Lombardi Cancer Center Tissue Culture Shared Resources. All cell lines were cultured as monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin, all obtained from Biosource (Rockville, MD) and incubated at 37°C in a 6% carbon dioxide humidified atmosphere. Doubling times of each cell line were assessed prior to treatments.

Cell Treatments

Cells were subjected to trypsin digestion for 1 minute (Sigma, St. Louis, MO), counted with a hemacytometer, and seeded at a density of 10^4 cells per well in 96-well plates. Cells were seeded for 12 hours in DMEM without FBS to synchronize cell cycles, and medium was changed to DMEM with FBS 12 hours prior to first treatment. A variety of treatment regimens were tested, varying from 1 to 3 treatments (1 every 4 hours) for 1 to 3 days, followed by 1 to 3 days recovery period. A range of succussed remedies were tested on each cell line, from 12 CH to 1000 CK potentization. Four of the final potentizations were performed in deionized ultra-filtered water (DiUF) from Fisher Scientific (Hampton, NH), with the final potentization prepared in cell culture medium. The alcohol content was by then minimized to 0.1 parts per billion. Controls included succussed and unsuccussed medium as well as DiUF vehicle in cell culture medium. Data are reported as percentage of unsuccussed medium used as control.

Crystal Violet

Crystal violet protein stain was performed on treated cell cultures for assessment of cell proliferation by total protein content. Briefly, cells were fixed and stained for 10 minutes with crystal violet (0.5%) from Fisher Scientific and then solubilized with citrate buffer (0.1 M). Color intensity was measured using a 96-well plate reader at 570 nm with a reference reading at 655 nm.

MTT

Cell viability was assessed using the MTT (3-(4,5-dimethylethiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. MTT was purchased from Invitrogen (Carlsbad, CA). Cells were incubated for 5 hours with 5 mg/mL MTT in phosphate buffered saline (PBS) at 37°C, 6% CO_2. The resulting formazan crystals were resuspended in dimethyl-sulfoxide from Fisher Scientific. Light absorption was measured using a 96-well plate reader at 570 nm with a reference reading at 655 nm.

Animal and Homeopathic Regimen

All animal procedures were carried out in accordance with the Georgetown University Department of Comparative Medicine guidelines. In vivo studies were performed in male nude BALB/c nu+ mice from Charles River (Boston, MA). Mice were housed 5 per cage in ambient temperature with controlled air, subjected to a 12-hour light-dark cycle, and received standard food and water ad libitum. The animal facility personnel handling the animals had no information about the experiments. Four- to five-week-old mice were allowed to adapt to animal facility conditions for 1 week prior

to subcutaneous inoculation with 10^6 cells of either PC-3 human prostate or MDA-MB-231 human breast cancer cell lines in 100 µL PBS on both sides of the abdomen. Two days postinoculation, mice were divided into 5 groups of 10 animals each, except for the untreated group that had 6 animals. The treatments were administered as follows:

Untreated control (UT) – inoculated mice left untreated and only handled for weight and tumor measurements

200 CH succussed water (DiUF 200 CH) – mice treated with 100 µL of 200 CH potentized water

Homeopathic *Sabal serrulata* (SS 200 CH) – mice treated with 100 µL of a 200 CH succussion (potentization) of *Sabal serrulata*

Homeopathic multitreatment (MT) – mice treated with 100 µL of homeopathic doses of various remedies on a 7-day cycle

Days 1 and 4 – *Thuja occidentalis* 1000 CK
Days 2 and 5 – *Conium maculatum* 1000 CK
Days 3 and 6 – *Sabal serrulata* 200 CH
Day 7 – carcinosin 1000 CK

The potencies used are based on unpublished experiments performed on rats.

Starting 2 days postinoculation, mice received daily treatment by gavage. The final 5 potentizations for succussed treatment were prepared daily in deionized water prior to gavage. Treatment continued for 5 weeks for a total of 35 treatments. Animals were followed up for an additional 5 weeks after final treatment, and weight measurements were recorded once per week.

Tumor Measurement

Once tumors were of a palpable volume, 3-dimensional measurements were recorded with a Vernier caliper by a veterinary technician blinded to the experiment. Animal weights were also recorded at the time of tumor volume measurements. The volume formula ($L \times W \times H$) was used to estimate relative tumor volume. In addition, all tumors were weighed upon dissection.

MRI

A representative mouse from 3 of the treatment groups was subjected to imaging procedures for visualization of tumors. Mice were anesthetized with 1.4% isoflurane and scanned using a Brookline 7 telsa MRI with a 7 cm coil, following a T1 weighted protocol with fat suppression. Twenty-four abdominal images per scan were obtained at 0.7 mm thickness, and tumor volume analysis was performed with ImageJ software available from the National Institutes of Health (Bethesda, MD) by summing the average of 3 hand-traced regions (tumor area) per tumor image.

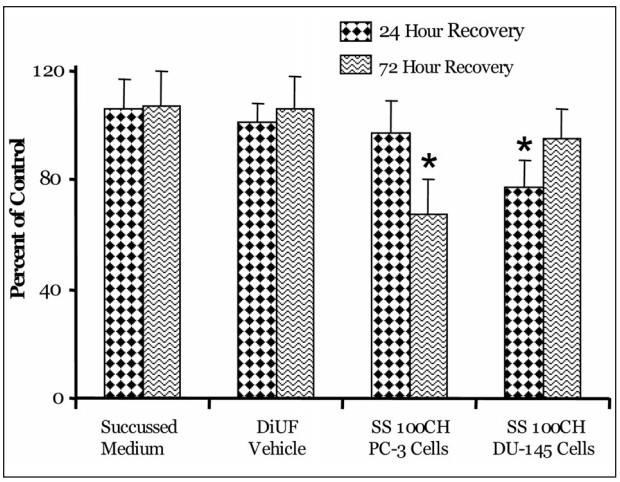

Figure 1 *Antiproliferative effect of 100 CH* Sabal serrulata *(SS 100CH) on both human prostate cancer cell lines PC-3 and DU-145. Cells were treated with 1 dose (10 µL) every 4 hours of* Sabal serrulata *and left to recover for either 24 or 72 hours. DiUF = succussed deionized ultrafiltered water; SS 100CH =* Sabal serrulata *100 CH dilution. Crystal violet technique was used. Error bars represent ± SD. PC-3, 72 hour and DU-145, 24 hour recovery are statistically significant,* $P < .01$ *(*) as assessed by Student's* t *test.*

Statistics

Following analysis of variance, Student's *t* test was performed to analyze the in vitro data. Repeated measures for analysis of variance (ANOVA) was used to test the significant difference between treatments in vivo. To assess the significant difference between treatments at each day point, ANOVA method was used. Statistical significance was considered only for *P* values less than .05.

Results

In vitro

Both human prostate and breast cancer cell lines were subjected to a battery of treatments with S*abal serrulata*, *Thuja occidentalis*, or *Conium maculatum* at different dilutions. After the last treatment, the cells were tested for proliferation either after 24 or 72 hours of recovery time. The proliferation was assessed using both crystal violet and MTT tests. The human prostate cancer cell lines responded differently to the various treatment settings. Following a 1-day regimen of 100 CH S*abal serrulata*, PC-3 cells showed reduced proliferation after a 72-hour recovery period. Cell proliferation was not significantly affected after 24 hours, but it was reduced by 33% after 72 hours. In contrast, DU-145 cells responded to an identical treatment after just a 24-hour recovery period (Figure 1). A 23% reduction was observed

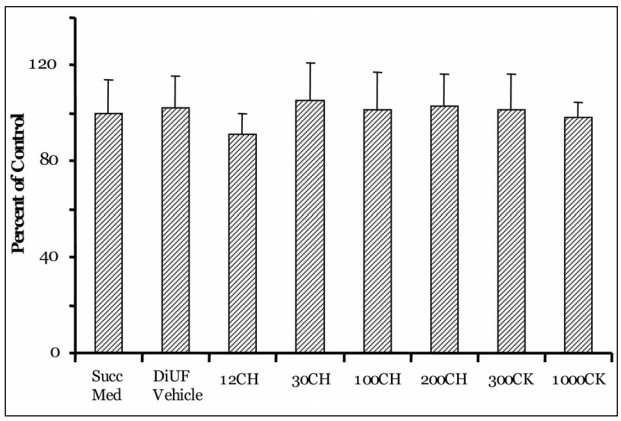

Figure 2 *Effect of increasing potency of* Sabal serrulata *preparations on human breast cancer MDA-MB-231 cell line. Cells were treated with 1 dose every 4 hours and left to recover 72 hours. Crystal violet method was used. DiUF = succussed deionized ultrafiltered water. Error bars represent ± SD. No statistically significant effect was observed,* P > .05.

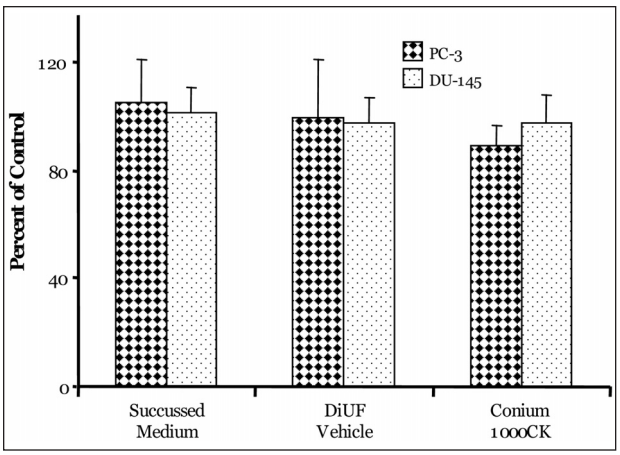

Figure 4 *Effect of 1000 CK* Conium maculatum *on both human prostate cancer cell lines PC-3 and DU-145. They were treated with 1 dose every 4 hours and left to recover for 24 hours. MTT technique was used. DiUF = succussed deionized ultrafiltered water. Error bars represent ± SD. No statistically significant effect was observed,* P > .05.

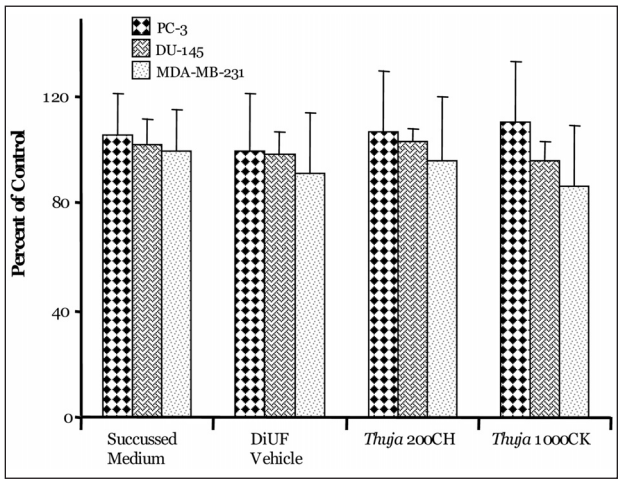

Figure 3 *Effect of 200 CH and 1000 CK* Thuja occidentalis *on both human prostate cancer cell lines PC-3 and DU-145 and human breast cancer MDA-MB-231 cells. They were treated with 1 dose every 4 hours and left to recover for 24 hours. Crystal violet method was used. DiUF = succussed deionized ultrafiltered water. Error bars represent ± SD. No statistically significant effect was observed,* P > .05.

after 24 hours, whereas no significant effect was noted after 72 hours. This difference is likely due to the specific proliferation rates—DU-145 cells grow faster than PC-3 cells—as tested by doubling time assessment (data not shown). Interestingly, when increasing potencies of *Sabal serrulata* ranging from 12 CH to 1000 CK were used, no significant cell proliferation inhibitory effect was observed on the human breast cancer cell line (Figure 2). Taken together, these findings indicate the specificity of *Sabal serrulata* to prostate cells.

Neither *Thuja occidentalis* nor *Conium maculatum* had an effect on cell proliferation of prostate cancer cells in vitro (Figures 3 and 4), or on breast cancer cells. Traditionally, these remedies are used to alleviate other distress-related symptoms that the patient may experience while suffering from prostate disturbances or prostate cancer.

In vivo

The mice were subjected to a regimen reflecting a homeopathic treatment that could potentially be recommended clinically by homeopaths to patients suffering from prostate problems including cancer. It is important to note that in this pilot study, 10 animals were used in each group except for the untreated control group, which was composed of only 6 animals. The smaller number of animals in the latter may have resulted in the observed intragroup variability. The tumor incidence rate for the prostate cancer untreated control group was at 100% by the end of the experiment, whereas it remained at 75% for the SS 200 CH, 94.5% for the MT, and 83% for the DiUF 200 CH. In the breast cancer group, the tumor incidence was between 90% and 100% in all animal groups. The treatments did not affect the body weights, as they remained the same throughout the experiment (Figure 5), and no toxicity due to the remedies was noticed. *Sabal serrulata* showed a more pronounced inhibitory effect on prostate cancer tumor growth than the combined regimen including *Thuja occidentalis*, *Conium maculatum*, and carcinosin (Figure 6). When compared to the untreated control group, tumor growth

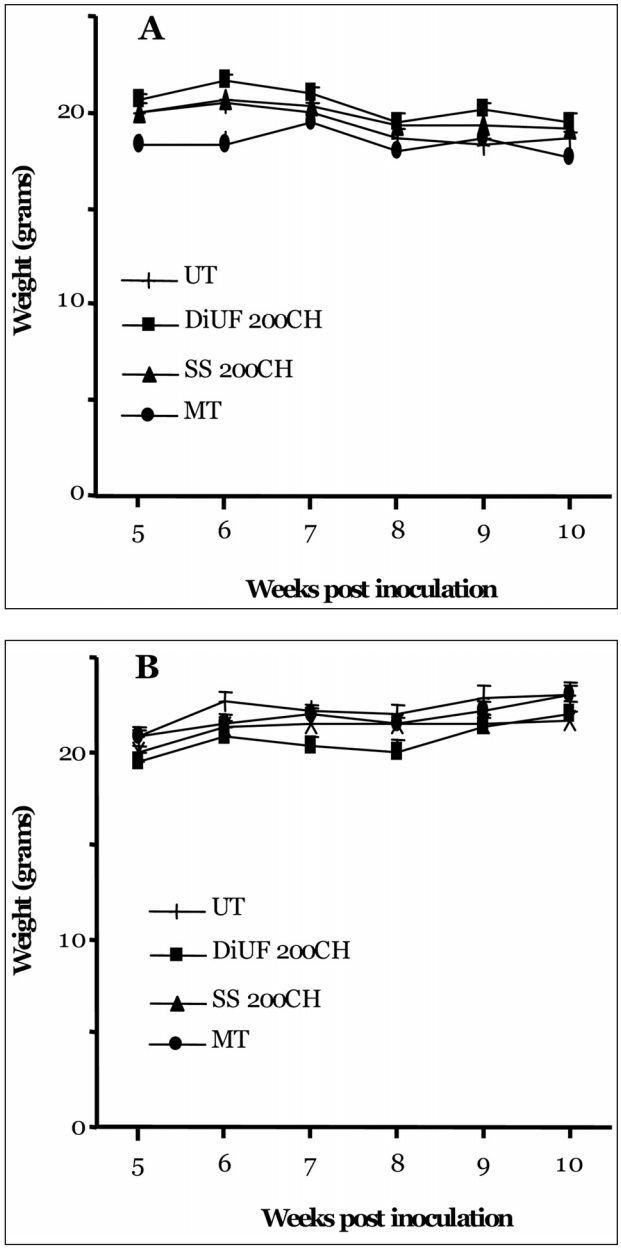

Figure 5 *Animal weights measurements. A, Represents the animal group inoculated with human prostate cancer PC-3 cells. B, Represents the animal group inoculated with human breast cancer MDA-MB-231 cells. UT = untreated; DiUF 200CH = deionized ultrafiltered water 200CH dilution; SS 200CH =* Sabal serrulata *200 CH dilution; MT = multitreatment. No significant differences were found between the various groups subjected to different treatments throughout the experiment time period.*

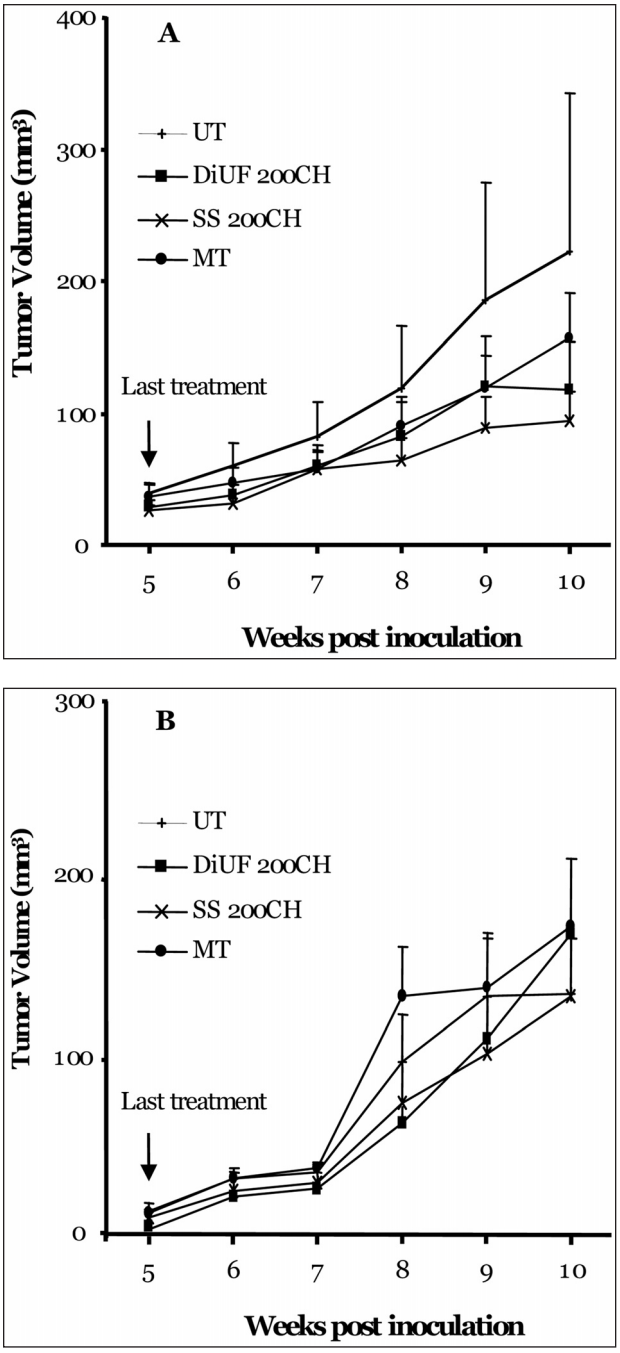

Figure 6 *Tumor growth assessment following 5-week treatment with homeopathic preparations. A, Tumor volumes measured in the animal group inoculated with human prostate cancer cells. By week 10, the mean tumor volume was reduced from 222 mm³ in the untreated control group to 94 mm³ in the SS 200CH group (*P = .012*) and to 117 mm³ in the DiUF 200CH group (*P = .048*). B, Represents the animal group inoculated with human breast cancer cell line. No significant differences were found (*P > .05*). UT = untreated; DiUF 200CH = deionized ultrafiltered water 200 CH dilution; SS 200CH =* Sabal serrulata *200 CH dilution; MT = multitreatment.*

in the *Sabal serrulata* treatment group was at 42% ($P = .012$), but this effect was marginally significant when S*abal serrulata* was administered biweekly as part of a regimen including *Thuja occidenatlis*, *Conium maculatum*, and carcinosin ($P = .07$). Animals treated with potentized deionized water showed reduced tumor growth as well when compared to untreated controls ($P = .048$). The fact that no effect on breast cancer tumor growth was observed using the same treatments

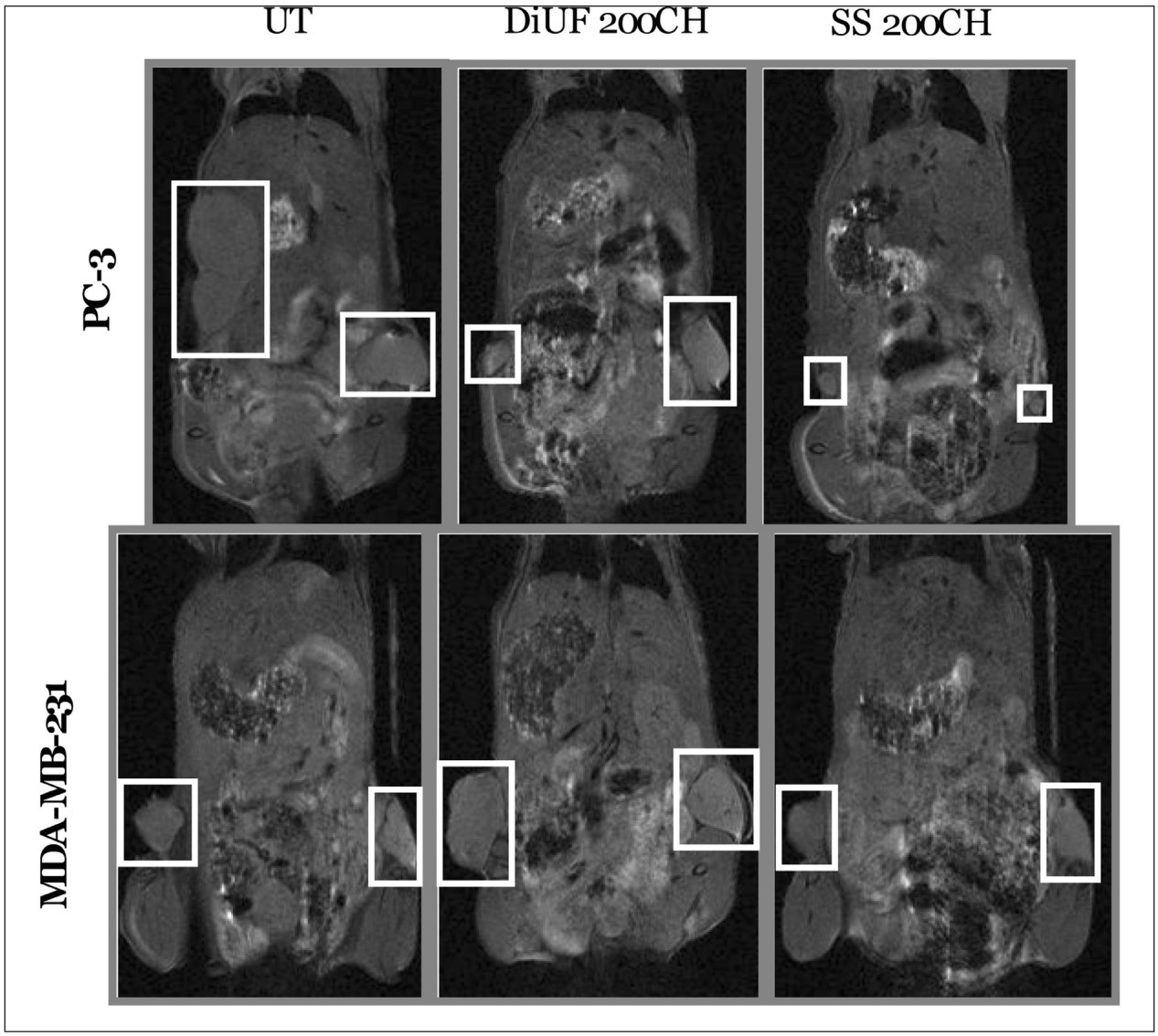

Figure 7 *Depiction of tumors developed in nude mice inoculated with either human prostate cancer (PC-3) or human breast cancer (MDA-MB-231) cells and subjected or not to various treatments. The delineated surface shows the tumor area. The animals were inoculated on both sides of the abdomen, which explains the growth on both sides of the animal. UT = untreated; DiUF 200CH = deionized ultrafiltered water 200 CH dilution; SS 200CH =* Sabal serrulata *200 CH dilution.*

(Figure 6B) indicates a certain prostate tissue specificity of *Sabal serrulata*'s effects. To capture tumor sizes by MRI, 1 representative animal from each group bearing the average size tumor underwent imaging. The smallest tumor size was observed in the prostate cancer animal group treated with homeopathic saw palmetto (Figure 7).

Discussion

Complementary and alternative therapies are used to prevent illness or side effects caused by conventional medical treatments, reduce stress, and control or cure disease. It is therefore not surprising that use of CAM therapies by cancer patients is on the rise. In a study published by Richardson and colleagues, 83% of 453 cancer patients had used at least one CAM therapy in their treatment.[32] Another survey reported that 37% of 46 patients with prostate cancer had used one or more CAM modalities.[33] These consisted mostly of herbal remedies, vitamins, and dietary measures. Although the German Commission E states that *Sabal serrulata* relieves symptoms associated with prostatic hypertrophy without reducing the enlargement itself,[34] homeopaths prescribe it to treat conditions involving both the female and male reproductive systems including prostate hypertrophy or atrophy accompanied by ulceration and bleeding. Using dilutions of 10^{-400}, 10^{-2000}, and $10^{-20\,000}$, Vozianov and Simeonova as

well as Pecherskii and colleagues studied the effects of homeopathic remedies on prostate adenoma and BPH. They suggested the interaction of the homeopathic medicine's informative energy imprint with the bioholographic human organism. Short- and long-term effects on both the target organ and higher regulatory centers indicated a definite therapeutic effect.[35,36] It is a challenge to understand and explain the mechanisms underlying the beneficial effects of homeopathic treatments. Therefore, most studies have focused on proving the biologic effects caused by homeopathic preparations in vitro and in vivo. Our work demonstrates the inhibitory effect of homeopathic *Sabal serrulata* on human prostate cancer cell line proliferation and on tumor growth.

By testing *Sabal serrulata* on human prostate cancer cell proliferation and comparing its effect to that on human breast cancer cell lines, we demonstrated that *Sabal serrulata* specifically inhibits prostate cancer cell proliferation. No effect was observed on the human breast cancer MDA-MB-231 cell line. Interestingly, the 2 prostate cancer cell lines PC-3 and DU-145 responded differently to *Sabal serrulata* treatment. PC-3 cells were not significantly affected after 24 hours, but their proliferation had been reduced by 33% at 72 hours. In contrast, a 23% reduction of DU-145 cell proliferation was observed after 24 hours whereas no significant effect was noted at 72 hours. This difference is likely due to the specific doubling times—DU-145 cells grow faster than PC-3 cells (data not shown)—and/or to the different ontogenic environments of the 2 cell lines, meaning that different cell-line-specific molecular mechanisms might have been triggered. Furthermore, the cell lines were also treated with homeopathic solutions of *Thuja occidentalis* and *Conium maculatum* with no significant effect on either human prostate or breast cancer cell lines.

Thuja occidentalis, also known as white cedar, has been traditionally used in folk medicine for various health conditions such as cystitis, prostate hypertrophy, urinary incontinence, uterine carcinoma, psoriasis, rheumatism, and condylomata.[37] Today, it is primarily used by homeopaths to treat a wide array of health problems ranging from warts to headaches, vertigo, emotional distress, depression, polydipsia, dysphagia, abdominal cramps, and so on. Its use for prostatitis is advised only if the symptoms are accompanied by urinary frequency, foam-covered cloudy urine, insomnia, and/or fever with chills that worsen in the evening. Several studies have identified various pharmacological properties of *Thuja occidentalis* as an herbal preparation. When applied to freshly infected MT-2 cells, *Thuja occidentalis* polysaccharides inhibit HIV-1-specific antigen expression in a dose-dependent manner.[38] Noteworthy among its immunopharmacological properties is its mitogenic potential triggering T-cell induction of CD4-positive T-helper/inducer cells with enhanced interleukin-2 (IL-2) production. In this case, *Thuja occidentalis* was shown to promote proliferation and differentiation of functional T-helper cells, an effect that was not observed with B cells.[39] Used with mouse macrophages, *Thuja occidentalis* induced an increase in IL-1, IL-6, and tumor necrosis factor-alpha (TNF-α). In addition to its effects on cytokine secretion, a stimulation of NO_2^- production was reported. The immunomodulatory potential of *Thuja occidentalis* has also been supported by animal studies.[40] In mice subjected to radiation, *Thuja occidentalis* treatment revealed protective properties against radiation toxicity.[41] Recently, Iwamoto and colleagues reported a potential antitumor effect of diterpenoids isolated from the stem of *Thuja standishii*.[42] Homeopathic tinctures of *Thuja occidentalis* have been evaluated for their genotoxic effects using the Salmonella/mammalian-microsome test and the induction of β-galactosidase, indicative of DNA damage. No mutagenic effects have been noted.[43]

Conium maculatum, commonly known as poison hemlock, is one of the most toxic species of the plant kingdom. Due to the narrow boundary between its therapeutic and toxic effects, its medicinal use remained very restricted. Besides its use as a fatal poison, the plant's seeds have been used as a sedative, antispasmodic, and analgesic. External application was directed at treating herpes, certain types of skin infection, and breast tumors.[44] *Conium maculatum* remains, however, a classic homeopathic remedy prescribed for enlarged and indurated immovable growths in glandular tissues occurring in aging patients. Thus, *Conium maculatum* is mostly used for breast cysts and tumors as well as prostate cancer. Within this symptomatic picture, homeopaths have reported successful breast and prostate cancer treatment.[45] Unfortunately, no basic science research has been conducted beyond the plant's powerful neurotoxic and teratogenic effects, and studies remain limited to the field of phytochemistry.[46] Our findings with regard to *Conium maculatum* in cellular and animal cancer models constitute the first report of its kind. In the above-described experimental setting, *Conium maculatum* did not show any toxicity. It did not affect the proliferation and viability of cells in vitro, and no toxicity was observed in mice. As a component of our chosen regimen, *Conium maculatum* did not significantly decrease tumor growth when compared to *Sabal serrulata* monotherapy.

Carcinosin is a nosode (a nosode is obtained from a diseased tissue or body secretions rather than animals, plants, or minerals) occupying a special place in homeopathy. Carcinosin could be viewed as a new therapeutic tool developed in the first part of the

twentieth century by W. Boericke, J. H. Compton Burnett, and J. H. Clarke, and subsequently revived by Dr D. M. Foubister in the 1950s.[47] It is traditionally prescribed for cancer patients presenting with an array of emotional problems, such as fear and low self-esteem. Depending on the preparation procedure, carcinosin could be obtained from a single tumor, as is the case for the Boiron and Dolisos brands in the United States, 15 tumors (Dolisos brand in Holland, Belgium, and Switzerland), or 58 tumors (Stauffen brand in Germany).[48] Carcinosin is therefore a new remedy awaiting not only full provings but also a standardized preparation procedure. Carcinosin 200 CH, a confirmed nosode of liver carcinoma used as monotherapy in *p*-dimethylaminoazo-benzene-induced hepatocarcinogenesis in mice treated daily until sacrifice, showed anticytotoxic, anticlastogenic, and some degree of antitumor effects.[26] In our experimental setting, carcinosin used once a week as part of a multitreatment regimen did not result in decreased tumor growth. The effect of the multitreatment regimen on tumor growth was in fact of the same magnitude as the succussed water effect. By week 10, which marked the end of the experiment, the most pronounced tumor growth reduction was observed with the monotreatment SS 200 CH, followed by succussed water (DiUF 200 CH) and the multitreatment regimen (MT). It would be of interest in future studies to observe the results of continuing treatment until the end of the experiment in order to mimic the clinically recommended homeopathic treatment approach. The observed treatment effect was sustained for 5 weeks after gavage cessation. This could indicate that homeopathy has a long-term effect and might act at the genomic level, targeting the underlying molecular mechanisms and pathways involved in the disease.

Deciphering the mechanistic aspects of the homeopathic effect is one of the most challenging endeavors in modern science. We observed that *Sabal serrulata* exerted its proliferative inhibitory effect specifically on human prostate cancer and that this effect was not obtained by using other remedies. This may suggest that the treatment's specificity is indicative of a cellular mechanism that remains yet to be elucidated. A potential cellular mechanism is suggested by the work of Gaddipati and colleagues, who observed growth inhibition of DU-145, PC-3, and LNCaP human prostate cancer cells following treatment with a pure compound derived from *Arnebia nobilis* root—Shikonin analog 93/637—at nonhomeopathic concentrations. Insulin-like growth factors (IGFs), known for their involvement in cell proliferation and their mitogenic effects, were impacted by that treatment. Thus, mRNA of IGF-I and IGF-IR was decreased in LNCaP, IGF-II in DU-145, and IGF-II and VEGF (vascular endothelial growth factor) in PC-3.[49]

Finding the best control for experimental and clinical research in homeopathy is an ongoing discussion. Most studies have been unable to assert the beneficial effect of homeopathy over control,[50,51] resulting in a number of explanatory theories. The potentization process, for example, the most enigmatic procedure in homeopathy, has been associated with the facilitation of a charged mineral-leak from the glass containers into the homeopathic solution, which in turn might exert an effect independent of the purported remedy.[52] Therefore, studies carried out with potentized controls will be more instructive. Investigating the influence of container materials and storage duration in glass and polyethylene containers on double distilled water and Argentum nitricum dissolved in double distilled water after potentization, as assessed by inductively coupled plasma-mass spectroscopy, Witt and colleagues demonstrated that element concentrations (Li, Na, Mg, Al, Si, Mn, Cu) were present to a higher degree in glass and already elevated during the first potentization step at the initial storage time.[53] Thus, the authors recommend the use of succussed controls, stored for the same time period and in the same type of container as their remedy counterparts. Using either nuclear magnetic resonance[54] or a biologic effect in mice,[55] a few reports documenting differences to this effect have been released. However, further attempts to demonstrate or replicate these differences[56] remained unsuccessful. In our study, we used succussed controls both in vitro and in vivo. There was a clear-cut difference in cell proliferation when *Sabal serrulata* was used as compared to a series of succussed and unsuccussed control solutions. In the in vivo study, by week 5, only 50% of animals receiving SS 200 CH developed tumors compared to 72% of the succussed water group, and 67% in the untreated group. These findings correspond to our experimental setting, that is, treatment for 5 weeks with 5 additional weeks of follow-up. The argument that compounds leaching from glass containers as a result of the potentization process might exert a therapeutic effect has created an intense debate in the scientific community. In future studies, alternate nonglass containers should be considered as an additional control.

Our study demonstrates a biologic response to homeopathic treatment as manifested by cell proliferation and tumor growth in cellular and animal models. This biologic effect was (i) significantly stronger to *Sabal serrulata* than to controls and (ii) specific to human prostate cancer. These data suggest *Sabal serrulata* as a homeopathic remedy of choice for prostate pathology. Repeating the in vivo pilot study will ensure experimental reproducibility of our findings and permit the application of modified protocols more

accurately reflecting the homeopathic therapeutic approach. In addition, new technologies capable of gene, protein, and pathway assessments in living organisms, such as functional genomics and proteomics, may elucidate the mechanisms underlying our observed antiproliferative and antitumorigenic effects. Controlled clinical studies would be needed to confirm this finding and assess *Sabal serrulata* as a remedy of choice.

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