Homeopathy in Experimental Cancer Models: A Systematic Review

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Homeopathy 2021;110:76–85.

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Abstract

Background Complementary and alternative medicine, including homeopathy, is widely used to improve well-being among cancer patients and reduce adverse effects of conventional treatment. In contrast, there are few studies on the use of homeopathic medicines to treat the disease itself. Yet, evidence of possible effectiveness of homeopathic high dilutions in experimental cancer models has been published during the past 20 years.

Aim The aim of the study was to perform a systematic review of fundamental research studies on homeopathic high dilutions in cancer.

Methods Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline, we conducted a literature search in the database PubMed for original publications, from 2000 to 2018 and in English, on *in vitro* and *in vivo* experimental cancer models testing homeopathic high dilutions.

Results Twenty-three articles met the inclusion criteria—14 *in vitro*, eight *in vivo*, and one *in vitro* plus *in vivo* experimental models. Most studies were from India. Research prominently focused on cytotoxic effects involving apoptotic mechanisms. Intrinsic aspects of homeopathy should be considered in experimental designs to emphasize the specificity of such effects.

Conclusion Fundamental research of homeopathy in cancer is still at an early stage and has mainly been performed by a few groups of investigators. The results point to an interference of well-selected homeopathic medicines with cell cycle and apoptotic mechanisms in cancer cells. However, these findings still need independent reproduction.

Keywords
- experimental oncology
- high dilutions
- methodological analysis

Introduction

Cancer is the second leading cause of death worldwide, accounting for about 9.6 million deaths in 2018, and one in six deaths in general.[1]

Many cancer patients around the world have recourse to traditional, complementary, and integrative medicine to improve their well-being and check the symptoms of adverse effects of conventional therapies.[2–7] Indeed, 8 of the 10 hospitals rated the best in 2019[8] provide such therapies, including adjuvant homeopathic treatment, with satisfactory outcomes.[9–13]

In contrast, there are almost no reports of homeopathic treatment of disease itself, and those available basically describe single cases or small series.[14,15] Facing this scenario, the experience of Banerjee et al in India stands out. Their results suggest that in addition to general stimulation of the immune system, homeopathic medicines also have tumor-specific action.[16,17] Such action, indeed, has been described in experimental studies since the early 2000s. However, to the best of

received
April 13, 2020
accepted after revision
June 15, 2020
published online
December 15, 2020

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

DOI https://doi.org/10.1055/s-0040-1716369.
ISSN 1475-4916.

our knowledge, just one single and difficult-to-access mini-review, of five pages only, has attempted a summary of all the available clinical and experimental evidence.[18] We were not able to locate any broad, encompassing review of experimental studies of homeopathic high dilutions in cancer.

Therefore, the aim of the present study was to perform a systematic review of experimental *in vivo* and *in vitro* studies of homeopathy in tumors and to identify methodological aspects that might need improvement. Based on our findings, we include some methodological recommendations for future studies.

Materials and Methods

For the present review we designed a protocol following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[19–21] We performed a literature search in the PubMed database with the following combinations of terms: homeopathy AND cancer AND oncology AND experimental; homeopathy AND tumor AND *in vitro*; homeopathy AND tumor AND *in vivo*; homeopathy AND cancer AND *in vivo*; homeopathy AND cancer AND *in vitro*.

Eligibility criteria were: experimental *in vivo* or *in vitro* studies of homeopathic (potentized) preparations in cancer models, including different designs and controls, published in English, from 2000 to the end of 2018. We located further records through hand search of references. We considered only original studies of high dilutions exclusively; reviews, surveys, opinions or comments were excluded. We also excluded clinical studies (in both human and veterinary medicine) and experimental studies with non-tumor cells alone.

Data considered were: author(s), year, country, rationale, homeopathic medicines tested (name, dilution(s) or potency, concentration in culture medium, length of exposure), animal species/cell lines, dosage, and outcomes. We also analyzed the methodological quality of studies based on number of repetitions, randomization, blinding, and use of controls, to estimate risk of bias.

The literature search was performed by one author as per the eligibility criteria. One author jointly analyzed pre-selected titles and abstracts for inclusion. Two authors extracted the relevant data and entered them on *ad hoc* tables for general findings and methodological quality of *in vivo* and *in vitro* studies separately. The results were then discussed and re-checked against the original data by two authors.

Results

We initially retrieved 96 records as per the search strategy. Forty-one records were duplicates, and a further two described the same experiments as in a third study and were considered as non-eligible. Following the analysis of titles and abstracts according to the inclusion/exclusion criteria, we included 23 full-text articles for analysis—14 reporting on *in vitro* models, eight on *in vivo* models, and one on both. Thirty-two records were excluded (►**Supplementary Files 1** and **2**, available online only).

The results are summarized as follows: ►**Table 1** (*in vitro*) and ►**Table 2** (*in vivo*) describe general findings (author(s), year, medicines, homeopathic dilutions [named as "potencies"], concentration, length of exposure, cell line, dosage, rationale, results, and adverse events). ►**Table 3** describes aspects related to the methodological quality of *in vitro* studies (repetitions, blinding, and controls) together with a brief analysis of weaknesses and strengths, and ►**Table 4** the methodological quality of *in vivo* studies (randomization, blinding, and controls). Therefore, ►**Tables 3** and **4** provide a measure of risk of bias among the analyzed studies.

One study[22] included both *in vitro* and *in vivo* models, the *in vitro* step having been performed to select the best medicine to be used *in vivo*. Since its main results concern the latter stage, we describe it together with the other *in vivo* studies. Interestingly, the results of both stages were convergent.

Overall, our analysis indicates that little experimental research has been done on homeopathy in cancer, and without any significant temporal trend. The largest proportion of studies was performed in India (13 of 22 studies, 63.6%), with a few contributions from the United States ($n = 3$), Brazil ($n = 2$), Switzerland ($n = 1$), and Turkey ($n = 1$). In addition, all three studies performed in the United States have Indian co-authors, and 10 of the 13 studies conducted in India were chaired by Khuda-Bukhsh.

Models, protocols and selected parameters follow the authors' research program/interests; thus the findings exhibit substantial heterogeneity that hinders drawing broad-scoped inferences. For this reason, in the next section we discuss the studies according to their group affiliation.

Several studies investigated direct anti-tumorigenic effects *in vivo* together with their possible mechanisms, including inhibition of cell proliferation, angiogenesis,[23] oxidative stress,[24,25] and gene and cytokine regulation—in the latter case, ruled out.[26]

In regard to methodological aspects, most authors did not report whether the studies were blinded or not—the same was the case for randomization among the *in vivo* studies. A large proportion of *in vitro* experiments comprised a single experiment, i.e., without any repetition. Controls varied considerably among the studies: most did not include negative and positive controls, but some included a comparator.

Finally, the two studies by Munshi et al[27,28] do not strictly address homeopathy in cancer, but used melanoma cells in the attempt to understand the mechanisms underlying homeopathic treatment of vitiligo. For this reason, we do not discuss them further here.

Discussion

A total of 23 records—12 *in vitro*, eight *in vivo*, and one with both *in vitro* and *in vivo* experimental models—met the inclusion/exclusion criteria and were considered for analysis. Such small number might be due to the rigorous eligibility criteria we established to ensure that only high-quality, peer-reviewed, and widely accessible studies would be considered for analysis.

Table 1 General description of *in vitro* experimental studies

First author/year	Country	Medicines, dilution, length of exposure	Cell line	Rationale	Main findings
Şeker et al 2018[32]	Turkey	Paclitaxel, docetaxel 6x, 5c, 15c 72 h.	Human breast cancer MCF-7.	Whether standard conventional anti-breast cancer medications retain activity when in homeopathic preparation	Continuity of biological actions in high dilution: changes in gene expression, concentration-independent; disruption of microtubule structure (target of taxanes)
Munshi et al 2019[27]	India	Kojic acid, hydrogen peroxide (HP), 6-biopterin, NLE 30c 48, 96 h.	Murine B16F10 melanoma.	Changes in melanin content of melanoma cells by vitiligo-producing substances.	No cytotoxic effect. Cells treated with NLE and HP exhibited higher melanin content compared to controls at 48 h; no effect at 96 h.
Khuda-Bukhsh et al 2017[40]	India	HIV nosode, 30c 24 h.	Human lung cancer A549; WRL-68 normal liver cells (control).	Viability of tumor vs. normal cells and involved mechanisms	Cytotoxic effect; reduced viability of tumor vs. normal cells. Mechanisms: prevented cancer cell proliferation and migration, induced premature senescence, enhanced pro-apoptotic signal proteins, inhibited anti-apoptotic signal proteins, changed mitochondrial membrane potential, caused externalization of phosphatidyl serine, membrane distortion, nuclear condensation, DNA fragmentation, and ROS generation.
Joshi et al 2017[28]	India	Hydroquinone (HQ), *Arsenicum sulphuratum flavum* (ASF), *Phosphorus* 30c 48, 96 h.	Murine B16F10 melanoma	Investigation of melanogenic activity of homeopathic medicines.	No cytotoxicity. Only HQ and ASF significantly increased melanin content compared to controls and vehicle. No inhibition of tyrosinase activity.
Nascimento et al 2016[46]	Brazil	CANOVA Variable according to test.	Human lymphocytes exposed to NMU.	Investigation of antigenotoxic effects	Significant reduction of NMU-induced DNA damage and induced apoptosis.
Wani et al 2016[48]	India	*Terminalia chebula* MT, 3x, 6c, 30c 24 h (viability) 24 – 72 h (cell growth).	Human cancer MDA-MB-231, MCF-7, HEK.	Viability of tumor vs. normal cells.	Cytotoxic effect: decreased viability and growth of tumor cells only.
Mondal et al 2016[41]	India	*Psorinum* 6x 24 h	Human lung A549, liver HepG2, breast MCF-7 cancer; WRL-68 liver non-cancer cells.	Pro-apoptotic mechanisms	Pro-apoptotic effects included cell cycle arrest, reduced mitochondrial activity, increased oxidative activity, enhanced pro-apoptotic signal proteins.
Sikdar et al 2014[42]	India	*Condurango* 6c, 30c 24–48 hours.	Human lung cancer NCI-H460	Comparison between dilutions below and above Avogadro's number on apoptosis and involved mechanisms.	Both dilutions were effective, 30c significantly more as asserted in homeopathic theory. Mechanisms: cell cycle arrest, altered expressions of certain apoptotic markers, ROS elevation, and MMP depolarization at 18–24 h
Samadder et al 2013[43]	India	*Lycopodium clavatum* 5c, 15c 24 h.	Human cervical cancer HeLa; normal PBMC (control).	Anti-cancer effects of *Lycopodium*; comparison between dilutions below and above Avogadro's number on apoptosis and involved mechanisms.	Cytotoxic effect: decreased viability of tumor cells only. Pro-apoptotic effect: DNA fragmentation, enhanced pro-apoptotic signal proteins, down-regulation of anti-apoptotic pathways. Variable differences between the 2 tested dilutions.
Bishayee et al 2013[44]	India	*Condurango* 30c 48 h	Human cervical cancer HeLa.		Cytotoxicity: striking reduction of HDAC2 activity.

Table 1 (*Continued*)

First author/year	Country	Medicines, dilution, length of exposure	Cell line	Rationale	Main findings
				Epigenetic modulation of histone-mediated cell cycle arrest.	
Arora et al 2013[64]	India	*Sarsaparilla*/human renal adenocarcinoma; ACHN + canine normal kidney cells MDCK; *Ruta graveolens*/colon carcinoma COLO 205; *Phytolacca decandra*/breast cancer MCF-7. MT, 30c, 200c, 1M, 10M 48 h.		Cytotoxicity of Banerjee protocol drugs/tumors.	Cytotoxicity and decreased proliferation of tumor cells only; greatest effect with MT, but remained in all the dilutions.
Mukherjee et al 2013[45]	India	*Thuja occidentalis* 30c 24 h.	BaP-intoxicated mice lung cells.	Protective role of *Thuja occidentalis* against normal lung cells exposed to lung carcinogen.	Increased viability of BaP intoxicated cells through down-regulation of ROS and HSP-60 and increased GSH context. No direct interaction with DNA, but striking ability to repair BaP-induced DNA damage. No effect on normal cells.
Frenkel et al 2010[49]	United States	*Carcinosinum* 30c, *Phytolacca decandra* 200c, *Conium maculatum* 3c, *Thuja occidentalis* 30c 24, 48, 72, 96 h.	Human breast cancer MCF-7, MDA-MB-231. Non-tumor human mammary epithelial cells HMLE.	Mechanism of action of Banerjee protocol drugs/tumors.	Preferential cytotoxic effects against the two breast cancer cell lines, causing cell cycle delay/arrest and apoptosis; effects were accompanied by altered expression of cell cycle regulatory proteins, and activation of the apoptotic cascade involving caspase 7 and PARP cleavage.
Wälchli et al 2006[50]	Switzerland	Cadmium chloride in potency pool (15–20c) 120 h	Human primary lymphocytes; acute T-cell leukemia (Jurkat) cells.	Hypothesis: primary cells are fitter to respond to high potencies than cell lines, especially cancer cell lines.	Increased viability of primary cells only; cancerous lymphocytes lost the ability to respond to regulatory signals and seemed unresponsive to high homeopathic potencies.

Abbreviations: BaP, benzo(a)pyrene; c, centesimal homeopathic dilution; GSH, total glutathione; HDAC, histone deacetylase; hsp, heat-shock protein; M, millesimal homeopathic dilution; MMP, mitochondrial membrane potential; MT, mother tincture; NLE, Nle4, D-Phe7]-α-melanocyte-stimulating hormone; NMU, *N*-methyl-*N*-nitrosourea; PARP, poly(ADP-ribose) polymerase; PBMC, peripheral blood mononuclear cells; ROS, reactive oxygen species; x, decimal homeopathic dilution.

The leading position of India in homeopathy research on cancer since the 2000s was evident also in this study and makes further comments unnecessary.[29,30]

The best effect on tumor reduction *in vitro* was obtained on prostate tumor cells with repeated administration of *Sabal serrulata*, which was confirmed *in vivo*.[22] In turn, Andrade et al described an innovative *in vivo* protocol[23] in which mice were continually exposed to a homeopathic complex (M1) for 14 days through a nebulizer, resulting in significant reduction of transplanted B16-F10 melanoma cells. These two studies point to a relevant issue to be considered in future experimental designs: in what measure are repeated doses necessary to achieve significant effects on tumor development? In addition, we should observe that Oliveira et al recently reproduced the same effects obtained by Andrade et al[23] in an *in vitro* model, adding a mechanistic molecular explanation to the observed effects in single B16-F10 cell cultures.[31]

In their study, Şeker et al[32] sought to identify continuity between the effect of taxanes as used in conventional treatment for breast cancer and the same drugs in high dilution. Besides a demonstration of effectiveness of high dilutions, it is very difficult to infer any application for this type of research, since effects were less significant with high dilutions compared to pharmacological dosage. In addition, the authors do not seem to have designed their study to verify some of the known features of high dilutions, such as linearity, non-linearity, or inversion of high dilution effects[33–35]—which might be an interesting contribution of further studies—since these topics are not considered in their discussion.

The group chaired by Khuda-Bukhsh has developed a consistent line of research during the analyzed 20 years. Their earliest work corresponds to *in vivo* studies to investigate liver anti-cancer effects of homeopathic medicines that have been long known in clinical practice for liver conditions, namely *Chelidonium majus* and *Lycopodium clavatum*. For this series of studies, these authors had recourse to a single model—*p*-DAB and PB-induced hepatocarcinogenesis in Swiss albino mice, and analyzed number and size of tumors, genotoxic potential, and activity of liver enzyme biomarkers, with significant effects among the treated animals compared to controls.[36–39] Next they sought to establish whether

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Table 2 General description of *in vivo* experimental studies

First author/ year	Country	Medicines, dilution, length of exposure	Model/rationale	Dosage	Main findings	Side effects
Andrade et al 2016[23]	Brazil	M1 homeopathic formula	Pulmonary metastatic and subcutaneous melanoma (B16F10) models/ B16F10/mice C57BL Potential benefits of M1 in melanoma.	M1 inhaled (10 minutes every 12 hours) for 14 days.	Lower tumor burden in the lungs and subcutaneous tissue than control mice; tumors were impaired in proliferation and angiogenesis.	No
Banerjee et al 2010[24]	India	*Chelidonium majus* 30c, 200c. 30, 60, 90, 120 days.	*p*-DAB and PB-induced hepatocellular carcinoma/white rats (*R. norvegicus*). Effectiveness of *Chelidonium majus* to prevent carcinogen-induced hepatotoxicity.	Twice per day, gavage.	Lower incidence of tumors; reduced activity of hepatotoxicity markers and oxidative stress.	No
Kumar et al 2007[25]	India	*Ruta graveolens, Hydrastis canadensis, Lycopodium clavatum, Thuja occidentalis* 20c, *Phosphorus* 1M.	*In vitro*: (1) *Ruta, Hydr, Lyc, Thuj* on NDEA-induced hepatocellular carcinoma/Wistar rats; (2) *Ruta, Phos* on 3-MC-induced sarcoma/Swiss albino mice. Corroboration of previously reported anti-cancer effects.	50 µL/animal/dose, 5 days/ week, gavage, concomitantly with carcinogens.	Reduced activity of hepatotoxicity markers and oxidative stress. Reduced incidence of 3-MC sarcomas and longer survival of tumor-harboring mice.	No
MacLaughlin et al 2006[22]	United States	*Sabal serrulata* 200c, *Thuja occidentalis* 1M, *Conium maculatum* 1M, Carcinosinum 1M. *In vitro*: 24–72 h *In vivo*: 7-day alternate drug protocol repeated over 5 weeks + 5-week post-treatment follow-up.	Preliminary *in vitro* experiment to select the best medicine: human prostate cancer PC-3 and DU-145; human breast cancer MDA-MB-231. *In vivo* experiment: PC-3, MDA-MB-231/ male nude BALB/c nu++ mice *Sabal* specificity in prostate cancer.	Alternate drug 7-day protocol over 5 weeks, gavage.	*In vitro*: *Sabal* induced 33% decrease of PC-3 cell proliferation at 72 hours and 23% reduction of DU-145 cell proliferation at 24 h. No effect on MDA-MB-231 breast cancer cells. *Thuj* and *Con* did not have any effect. *Sabal* was selected for *in vivo* phase. *In vivo*: significant reduction of prostate tumor with *Sabal* compared to untreated controls; no effect on breast tumor growth.	Not reported
Thangapazham et al 2006[26]	United States	*Conium maculatum, Sabal serrulata, Thuja occidentalis, Asterias rubens, Phytolacca decandra* 30c, 200c, 1M MAT-LyLu nosode 1M.	MAT-LyLu prostate cancer/Copenhagen rats. Effect of homeopathic treatment on the expression of genes involved in apoptosis and cytokines in tumor and lung tissue and mechanisms.	100 µL/day; 7-day alternate drug protocol over 5 weeks.	Significant reduction in the tumor incidence (23%), tumor volume (45%) and tumor weight (33%). Effects not explained by changes in pro-apoptotic genes or cytokines in prostate tumor or lung metastasis.	Not reported
Pathak et al 2006[36]	India	*Lycopodium clavatum* 30c 7, 15, 30c, 60, 90, and 120 days.	*p*-DAB-induced hepatocarcinogenesis/ Swiss albino mice. Test liver anti-cancer properties of *Lycopodium clavatum*, widely used for hepatobiliary disorders.	0.06 mL twice a day until euthanasia.	No or fewer tumors; reduced genotoxicity; reduced activity of liver toxicity markers.	Not reported
Biswas et al 2005[37]	India	*Chelidonium majus* and *Carcinosinum* 200c alone and combined 7, 15, 30, 60, 90, 120 days.	*p*-DAB-induced hepatocarcinogenesis/ Swiss albino mice. Whether *Carcinosinum* may enhance the anti-cancer effect of *Chelidonium majus*.	0.06 mL *Chel*: twice a day until euthanasia; *Carc*: once a day until euthanasia.	One or both remedies, alone or in combination, reduced the number and size of tumors, reduced genotoxicity and activity of hepatotoxicity biomarkers.	No

Table 2 (*Continued*)

First author/ year	Country	Medicines, dilution, length of exposure	Model/rationale	Dosage	Main findings	Side effects
Biswas & Khuda-Bukhsh 2004[38]	India	*Chelidonium majus* 30c, 200c.	*p*-DAB and/or PB-induced hepatocarcinogenesis/Swiss albino mice. Investigation of liver anti-cancer effect of *Chelidonium majus*, widely used for liver diseases.	0.06 mL three times/day 7 days, then twice a day until euthanasia.	Reduced number of tumors and genotoxicity; favorable modulation of toxicity marker enzymes.	No
Biswas & Khuda-Bukhsh 2002[39]	India	*Chelidonium majus* 30c, 200c.	*p*-DAB and/or PB-induced hepatocarcinogenesis/Swiss albino mice. Investigation of liver anti-cancer effect of *Chelidonium majus*, widely used for liver diseases.	0.06 mL three times/day 7 days, then twice a day until euthanasia.	Reduced number of tumors and genotoxicity; favorable modulation of toxicity marker enzymes; mild splenomegaly.	No

Abbreviations: 3-MC, 3-methylcholantrene; c, centesimal homeopathic dilution; M, millesimal homeopathic dilution; NDEA, *N*-nitrosodiethylamine; PB, phenobarbital; *p*-DAB, *p*-dimethyl amino azo benzene.

Carcinosinum, i.e., the cancer nosode, could enhance these anti-cancer effects.[37]

The studies performed by this group in the 2010s had a new and different focus: to document pro-apoptotic effects of homeopathic medicines on cancer cells and establish their underlying mechanisms.[40–45] In all these cases, they reported positive findings—cytotoxic action, involving several signal proteins, the mitochondrial membrane potential, oxidative activity, cell cycle arrest, and DNA damage. These findings agree with those reported by Nascimento et al[46] for CANOVA—a formula that combines the homeopathic medicines *Aconitum napellus, Arsenicum album, Bryonia alba, Lachesis muta* and *Thuja occidentalis* in dilutions 11x to 19 x .[47]

The hypothesis that homeopathic medicines only hinder the viability of tumor cells, without any cytotoxic activity on normal ones, was tested by Wani et al,[48] and Frenkel et al,[49] both of which groups analyzed drugs and cancer models included in the Banerjee protocol. Against this evidence, however, Wälchli et al[50] hypothesized and demonstrated that primary cells are fitter to respond to high potencies than cell lines, especially cancer cell lines. The alleged reason is that cancer cells lose the ability to respond to regulatory signals, and thus might become unresponsive to subtle stimuli such as those represented by homeopathic high dilutions.

The study by MacLaughlin et al[22] stands out as regards anti-tumorigenic effects *in vivo*. *Sabal serrulata* (*Serenoa repens*) has long been known for its effects on benign prostatic hyperplasia, both in pharmacological and in homeopathic doses.[34,47] These authors sought to establish whether this medicine has, indeed, preferential action in the prostate. Therefore, they tested its effects on prostate and breast cancer *in vivo* and *in vitro* and compared the effects on the former to other homeopathic medicines widely indicated for tumors in general (*Conium maculatum*, *Thuja occidentalis*, *Carcinosinum*). The results confirmed their hypothesis and are even more robust since there was agreement between the *in vitro* and *in vivo* findings.

The variability we found in selected controls among the analyzed studies points to a very serious issue in high dilution research: namely, what comprises ideal controls. In principle, positive and negative controls are assumed to be the best parameters to establish the presence and degree of a certain effect: for instance, cytotoxicity against tumor cells *in vitro*. However, some variables are critical in the case of high dilution research, even though they are irrelevant in conventional pharmacological studies. For example, using non-succussed water or untreated cells may hide eventual non-specific effects resulting from succussion, such as leaching from flask walls[51] and generation of nanobubbles,[52] which might change several physico-chemical parameters of water or other vehicles.[53–55] This point is critical for *in vitro* but less relevant for *in vivo* studies, due to the complex interaction of high dilutions with gastrointestinal tissues. In some cases, this interaction is not even needed.[23] In turn, blinding and randomization of animals are fundamental methodological criteria *in vivo*. Researchers and animals systematically interact during experimental procedures, which may act as a potential cause of bias. As ►**Table 4** shows, only one of nine studies reported randomization and two of nine reported blinding.

Table 3 Methodological quality of *in vitro* experimental studies

First author/year	Controls	Strengths	Weaknesses
Şeker et al 2018[32]	Untreated cells (negative); undiluted alcohol (named placebo); tested drugs in pharmacological concentration (positive).	Three repetitions; positive and negative controls.	No blinding; no rationale for testing diluted taxanes.
Munshi et al 2019[27]	Potentized and non-potentized alcohol.	Three repetitions	No blinding
Khuda-Bukhsh et al 2017[40]	Cells treated with potentized ethanol from the same stock.	All experiments were done in triplicate and replicated thrice; multiple experiments on same model.	Blinding not reported; no rationale for action of HIV nosode in lung cancer.
Joshi et al 2017[28]	Potentized and non-potentized alcohol.		No repetitions; blinding not reported.
Nascimento et al 2016[46]	Non-exposed untreated cells (negative); exposed untreated cells (positive); non-exposed treated cells; exposed treated cells.		Blinding not reported; no repetitions.
Wani et al 2016[48]	40 to 5% alcohol (pre-test); untreated cells (negative). The dilutions of alcohol to test its toxicity (pre-test) were 1: 2.5 up to 1:20.	Compared cancer and non-cancer cells; alcohol toxicity in different concentrations; toxicity investigated in pre-test; nanoparticle analysis.	No repetitions; no blinding.
Mondal et al 2016[41]	Alcohol 6x	Choice of sensitive cell line in pilot study; several assays to elucidate mechanisms.	No repetitions; no blinding; rationale based only on clinical empirical data.
Sikdar et al 2014[42]	Vehicle	Blinding; several analysis methods including key proteins in apoptosis and cell morphology.	No repetitions
Samadder et al 2013[43]	Untreated HeLa cells (negative); treatment with vehicle (placebo, positive); conventional chemotherapy agent (positive); PBMC (comparison).	Comparison of cancer and non-cancer cells; positive, negative, and comparison controls.	No repetitions; no blinding.
Bishayee et al 2013[44]	Non-potentized alcohol.	Blinding	No repetitions
Arora et al 2013[64]	Untreated cells; vehicle.	Comparison of cancer and non-cancer cells.	No repetitions; no blinding.
Mukherjee et al 2013[45]	Untreated cells (positive); vehicle (negative).	Three repetitions; positive and negative controls; simultaneous analysis of several parameters.	No blinding
Frenkel et al 2010[49]	Untreated cells; vehicle.	Treatments at different times and different cells lines; simultaneous analysis of several apoptosis and oncogenesis aspects.	No repetitions; no blinding.
Wälchli et al 2006[50]	Pool de-ionized water potencies (15–20c).	Partial blinding; cells challenged with several concentrations of the toxic agent.	No repetitions; one single parameter of analysis.

Abbreviations: Potentized, submitted to serial dilution and agitation following homeopathic technique; PBMC, peripheral blood mononuclear cells.

Again, as concerns controls, the use of succussed alcohol is a subject of controversy. Diluted and agitated alcohol is no mere "potentized vehicle", but a proper homeopathic medicine—*Alcoholus* or *Ethylicum*—with its specific set of signs and symptoms obtained in homeopathic pathogenetic trials.[56] In addition, some authors reported specific effects of potentized alcohol in experimental models *in vitro* by comparison to other controls.[57,58] Therefore, potentized alcohol seems to be more of a comparator than a negative control. Non-potentized alcohol may be used instead, or together with succussed water. A recent experimental *in vitro* study showed how important is it to characterize non-specific effects of vehicles.[59] From this perspective, using both succussed and non-succussed vehicles may represent a good standard for controls in future studies *in*

Table 4 Methodological quality of *in vivo* experimental studies

First author/year	Randomization	Blinding	Controls
Andrade et al 2016[23]	Not reported	Yes	Animals treated with vehicle.
Banerjee et al 2010[24]	Yes	Yes	Untreated animals (negative); potentized alcohol; animals treated with *p*-DAB (positive); animals treated with *p*-DAB and potentized alcohol.
Kumar et al 2007[25]	Not reported	No	Potentized alcohol; untreated, unchallenged animals (healthy).
MacLaughlin et al 2006[22]	Not reported	No	Untreated, unchallenged animals; potentized water.
Thangapazham et al 2006[26]	Not reported	No	Animals treated with potentized water.
Pathak et al 2006[36]	Not reported	No	Unexposed untreated; unexposed treated with potentized vehicle; exposed untreated; exposed treated with potentized vehicle; exposed treated with *Lyc*.
Biswas et al 2005[37]	Not reported	No	Animals treated with vehicle.
Biswas & Khuda-Bukhsh 2004[38]	Not reported	No	Animals treated with vehicle.
Biswas & Khuda-Bukhsh 2002[39]	Not reported	No	Untreated animals.

Abbreviation: DAB, dimethyl amino azo benzene.

vitro. As shown in ►**Table 3**, only two studies used both types[27,28] whilst most only used vehicle and untreated cells, following the standards of conventional pharmacological research.

The choice of medicines and potencies, as well as their relationship to the main effect, is still unclear. In addition, the possibility of improving outcomes through a combination of potencies (known as "potency chord")[60] is still an open field for further investigation. One single study implemented this experimental design.[50]

Based on our findings, we offer the following recommendations for future studies:

1. *Rationale*: Investigators should have a clear idea of what they intend to demonstrate in each study and what implications are in terms of biological and pharmacological theory. As is universally known, fundamental research usually does not generate findings with immediate practical applications. Therefore, investigators should not assume that their results will in any way contribute to the actual treatment of cancer patients, human or animal, although this might be the main motivation for studies. No *in vitro* study can be used to validate empirical uses of some medicines, considering that the tissue micro-environment is a crucial factor in tumor progression.[60] *In vivo* studies and clinical trials are mandatory to validate new therapies, provided they systematically comply with ethical standards. Citations used to ground experiments should be duly screened and checked for reliability. When reporting conclusions, investigators should clearly state what the implications of their study are for the state of the art and how it may contribute to future research.
2. *Blinding and randomization*: Both are essential requirements in scientific research of homeopathic high dilutions to reduce bias, since these medicines' mechanism of action is still unknown, and many unexpected variables may interfere somehow with the results. These two procedures,[61] plus allocation concealment, should be systematically reported.
3. *Statistical analysis*: All care should be taken to include the due statistical analysis of results. In many of the analyzed studies, for instance, there was no indication of the statistical significance of findings (e.g., number of tumors between treated and untreated groups).
4. *Controls*: All studies, without any exception, should include positive and negative controls, which should be clearly described in a separate sub-section. Study designs with different vehicle conditions (succussed water and non-succussed alcohol) must be considered since the agitation seems to be crucial to reveal any effect of homeopathic medicines. However, it can also produce several physical changes in water or other solvents likely to induce non-specific effects.[59]
5. *Alcohol*: Alcohol is not at inert substance in either pharmacological dose or homeopathic preparation.[62] As in the case of the former, any effect of potentized alcohol cannot be considered a non-specific "vehicle effect", but a specific effect of the homeopathic medicine *Alcoholus/Ethylicum*. In addition, all studies on cells should include a pre-test of alcohol cytotoxicity or a specific citation on this issue. We further suggest preparing final working potencies in sterile water from conventional hydro-alcoholic solutions.[63]
6. *Repetitions*: All *in vitro* experiments yield more reliable outcomes when they are repeated and average results of independent series of tests are reported. Even better, they should be reproduced by different laboratories, or tested in multi-center studies.

Conclusion

Fundamental research of homeopathy in cancer is still at an early stage and mainly performed by single groups of investigators, mostly from India. Results should be reproduced at different laboratories and also in other countries.

Our results point to an interference of homeopathic high dilutions with the cell cycle and apoptotic mechanisms in

cancer cells. The available evidence suggests that well-selected homeopathic medicines have preferential cytotoxic action on cancer versus normal cells.

One of the analyzed studies had a broad encompassing design, based on a solid clinical background, and with coherent results observed in both *in-vivo* and *in-vitro* experiments: it may serve as a model for further initiatives. Additional methodological refinement based on specific aspects of high dilutions is necessary, such as the choice of the best controls.

Highlights

- Effectiveness of homeopathic products on cancer has been experimentally tested since the early 2000s.
- A systematic review of these studies was performed using PRISMA methods.
- Cell cycle arrest and increase in apoptosis rate were the most reported findings.
- Methodological deficiencies were revealed among the studies.
- Recommendations for further studies are suggested.

Supplementary Files

Supplementary File 1 PRISMA flowchart of the study design.
Supplementary File 2 List of excluded studies according to the exclusion criteria.

Conflict of Interest
None declared.

Acknowledgments/Funding
The authors would like to thank Universidade Paulista for the PhD grants to Andreza Pereira dos Santos and Thayná Neves Cardoso. The study had no other funding source.

References

1 World Health Organization. Cancer, key facts (2018). Available at: https://www.who.int/news-room/fact-sheets/detail/cancer. Accessed April 5, 2020
2 Rostock M, Naumann J, Guethlin C, Guenther L, Bartsch HH, Walach H. Classical homeopathy in the treatment of cancer patients—a prospective observational study of two independent cohorts. BMC Cancer 2011;11:19
3 Fulop JA, Grimone A, Victorson D. Restoring balance for people with cancer through integrative oncology. Prim Care 2017; 44:323–335
4 Rossi E, Di Stefano M, Firenzuoli F, Monechi MV, Baccetti S. Add-on complementary medicine in cancer care: evidence in literature and experiences of integration. Medicines (Basel) 2017;4:5
5 Rossi E, Noberasco C, Picchi M, et al. Complementary and alternative medicine to reduce adverse effects of anti-cancer therapy. J Altern Complement Med 2018;24:933–941
6 Bosacki C, Vallard A, Gras M, et al. Les médecines alternatives complémentaires en oncologie. Bull Cancer 2019;106:479–491
7 Kacel EL, Pereira DB, Estores IM. Advancing supportive oncology care via collaboration between psycho-oncology and integrative medicine. Support Care Cancer 2019;27:3175–3178
8 Newsweek. World's best hospitals 2019. Available at: https://www.newsweek.com. Accessed April 4, 2020
9 Karp JC, Sanchez C, Guilbert P, Mina W, Demonceaux A, Curé H. Treatment with Ruta graveolens 5CH and Rhus toxicodendron 9CH may reduce joint pain and stiffness linked to aromatase inhibitors in women with early breast cancer: results of a pilot observational study. Homeopathy 2016;105:299–308
10 Sorrentino L, Piraneo S, Riggio E, et al. Is there a role for homeopathy in breast cancer surgery? A first randomized clinical trial on treatment with *Arnica montana* to reduce post-operative seroma and bleeding in patients undergoing total mastectomy. J Intercult Ethnopharmacol 2017;6:1–8
11 Bagot JL, Delègue C. My best case: homeopathic management of adverse effects of tamoxifen. Wien Med Wochenschr 2020; 170:224–229
12 Gaertner K, Lüer SC, Frei-Erb M, von Ammon K. Complementary individual homeopathy in paediatric cancer care: a case series from a University Hospital, Switzerland. Complement Ther Med 2018;41:267–270
13 Samuels N, Freed Y, Weitzen R, et al. Feasibility of homeopathic treatment for symptom reduction in an integrative oncology service. Integr Cancer Ther 2018;17:486–492
14 Rajendran ES. Homeopathy as a supportive therapy in cancer. Homeopathy 2004;93:99–102
15 Nwabudike LC. Homeopathy as therapy for mycosis fungoides: case reports of three patients. Homeopathy 2019;108:277–284
16 Bell IR, Sarter B, Koithan M, et al. Integrative nanomedicine: treating cancer with nanoscale natural products. Glob Adv Health Med 2014;3:36–53
17 Frenkel M. Is there a role for homeopathy in cancer care? Questions and challenges. Curr Oncol Rep 2015;17:43
18 Yadav R, Jee B, Rao KS. How homeopathic medicine works in cancer treatment: deep insight from clinical to experimental studies. J Exp Ther Oncol 2019;13:71–76
19 PRISMA. Transparent reporting of systematic reviews and meta-analyses. Available at: prisma-statement.org. Accessed April 4, 2020
20 Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 2009;6:e1000100
21 Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097
22 MacLaughlin BW, Gutsmuths B, Pretner E, et al. Effects of homeopathic preparations on human prostate cancer growth in cellular and animal models. Integr Cancer Ther 2006;5:362–372
23 Ferrari de Andrade L, Mozeleski B, Leck AR, et al. Inhalation therapy with M1 inhibits experimental melanoma development and metastases in mice. Homeopathy 2016;105:109–118
24 Banerjee A, Pathak S, Biswas SJ, et al. Chelidonium majus 30C and 200C in induced hepato-toxicity in rats. Homeopathy 2010;99: 167–176
25 Kumar KB, Sunila ES, Kuttan G, Preethi KC, Venugopal CN, Kuttan R. Inhibition of chemically induced carcinogenesis by drugs used in homeopathic medicine. Asian Pac J Cancer Prev 2007;8:98–102
26 Thangapazham RL, Rajeshkumar NV, Sharma A, et al. Effect of homeopathic treatment on gene expression in Copenhagen rat tumor tissues. Integr Cancer Ther 2006;5:350–355
27 Munshi R, Joshi S, Talele G, Shah R. An in-vitro assay estimating changes in melanin content of melanoma cells due to ultra-dilute, potentized preparations. Homeopathy 2019;108:183–187
28 Joshi S, Munshi R, Talele G, Shah R. Evaluation of melanogenic and anti-vitiligo activities of homeopathic preparations on

murine B16-F10 melanoma cells. Eur J Pharm Med Res 2017;4: 718–723
29 Klein SD, Würtenberger S, Wolf U, Baumgartner S, Tournier A. Physicochemical investigations of homeopathic preparations: a systematic review and bibliometric analysis – part 1. J Altern Complement Med 2018;24:409–421
30 Waisse S. Effects of high dilutions on in vitro models: literature review. Rev Homeopatia 2017;80:90–103
31 Gonçalves JP, Potrich FB, Ferreira Dos Santos ML, et al. In vitro attenuation of classic metastatic melanoma-related features by highly diluted natural complexes: molecular and functional analyses. Int J Oncol 2019;55:721–732
32 Şeker S, Güven C, Akçakaya H, Bahtiyar N, Akbaş F, Onaran İ. Evidence that extreme dilutions of paclitaxel and docetaxel alter gene expression of in vitro breast cancer cells. Homeopathy 2018; 107:32–39
33 Bellavite P, Signorini A, Marzotto M, Moratti E, Bonafini C, Olioso D. Cell sensitivity, non-linearity and inverse effects. Homeopathy 2015;104:139–160
34 Boericke W. Pocket manual of homoeopathic materia medica. New York, NY: Boericke & Runyon; 1922
35 Seligmann IC, Lima PDL, Cardoso PCS, et al. The anti-cancer homeopathic composite "Canova Method" is not genotoxic for human lymphocytes in vitro. Genet Mol Res 2003;2:223–228
36 Pathak S, Kumar Das J, Jyoti Biswas S, Khuda-Bukhsh AR. Protective potentials of a potentized homeopathic drug, Lycopodium-30, in ameliorating azo dye induced hepatocarcinogenesis in mice. Mol Cell Biochem 2006;285:121–131
37 Biswas SJ, Pathak S, Bhattacharjee N, Das JK, Khuda-Bukhsh AR. Efficacy of the potentized homeopathic drug, Carcinosin 200, fed alone and in combination with another drug, Chelidonium 200, in amelioration of *p*-dimethylaminoazobenzene-induced hepatocarcinogenesis in mice. J Altern Complement Med 2005;11: 839–854
38 Biswas SJ, Khuda-Bukhsh AR. Evaluation of protective potentials of a potentized homeopathic drug, *Chelidonium majus*, during azo dye induced hepatocarcinogenesis in mice. Indian J Exp Biol 2004; 42:698–714
39 Biswas SJ, Khuda-Bukhsh AR. Effect of a homeopathic drug, Chelidonium, in amelioration of *p*-DAB induced hepatocarcinogenesis in mice. BMC Complement Altern Med 2002;2:4
40 Khuda-Bukhsh AR, Mondal J, Shah R. Therapeutic potential of HIV nosode 30c as evaluated in A549 lung cancer cells. Homeopathy 2017;106:203–213
41 Mondal J, Samadder A, Khuda-Bukhsh AR. Psorinum 6x triggers apoptosis signals in human lung cancer cells. J Integr Med 2016; 14:143–153
42 Sikdar S, Kumar Saha S, Rahman Khuda-Bukhsh A. Relative apoptosis-inducing potential of homeopathic Condurango 6c and 30c in H460 lung cancer cells in vitro: apoptosis-induction by homeopathic Condurango in H460 cells. J Pharmacopuncture 2014;17:59–69
43 Samadder A, Das S, Das J, Paul A, Boujedaini N, Khuda-Bukhsh AR. The potentized homeopathic drug, *Lycopodium clavatum* (5C and 15C) has anti-cancer effect on HeLa cells in vitro. J Acupunct Meridian Stud 2013;6:180–187
44 Bishayee K, Sikdar S, Khuda-Bukhsh AR. Evidence of an epigenetic modification in cell-cycle arrest caused by the use of ultra-highly-diluted Gonolobus condurango extract. J Pharmacopuncture 2013;16:7–13
45 Mukherjee A, Boujedaini N, Khuda-Bukhsh AR. Homeopathic Thuja 30C ameliorates benzo(a)pyrene-induced DNA damage, stress and viability of perfused lung cells of mice in vitro. J Integr Med 2013;11:397–404
46 Nascimento HFS, Cardoso PCDS, Ribeiro HF, et al. In vitro assessment of anticytotoxic and antigenotoxic effects of CANOVA(®). Homeopathy 2016;105:265–269
47 Champault G, Patel JC, Bonnard AM. A double-blind trial of an extract of the plant *Serenoa repens* in benign prostatic hyperplasia. Br J Clin Pharmacol 1984;18:461–462
48 Wani K, Shah N, Prabhune A, Jadhav A, Ranjekar P, Kaul-Ghanekar R. Evaluating the anti-cancer activity and nanoparticulate nature of homeopathic preparations of *Terminalia chebula*. Homeopathy 2016;105:318–326
49 Frenkel M, Mishra BM, Sen S, et al. Cytotoxic effects of ultra-diluted remedies on breast cancer cells. Int J Oncol 2010; 36:395–403
50 Wälchli C, Baumgartner S, Bastide M. Effect of low doses and high homeopathic potencies in normal and cancerous human lymphocytes: an in vitro isopathic study. J Altern Complement Med 2006;12:421–427
51 Dalboni LC, Coelho CP, Palombo Pedro RR, et al. Biological actions, electrical conductance and silicon-containing microparticles of *Arsenicum album* prepared in plastic and glass vials. Homeopathy 2019;108:12–23
52 Demangeat JL. Gas nanobubbles and aqueous nanostructures: the crucial role of dynamization. Homeopathy 2015;104:101–115
53 Agarwal A, Ng WJ, Liu Y. Principle and applications of microbubble and nanobubble technology for water treatment. Chemosphere 2011;84:1175–1180
54 Agmon N, Bakker HJ, Campen RK, et al. Protons and hydroxide ions in aqueous systems. Chem Rev 2016;116:7642–7672
55 Yinnon TA. Liquids prepared by serially diluting and vigorously shaking of aqueous solutions: unveiling effects of the solute on their properties. Water 2020;10:115–134
56 Allen T. The Encyclopedia of Pure Materia Medica. New York, NY: Boericke & Tafel; 1877
57 Lima LF, Rocha RMP, Duarte ABG, et al. Unexpected effect of the vehicle (grain ethanol) of homeopathic FSH on the in vitro survival and development of isolated ovine preantral follicles. Microsc Res Tech 2017;80:406–418
58 Sandri PF, Portocarrero AR, Ciupa L, et al. Dynamized ethyl alcohol improves immune response and behavior in murine infection with *Trypanosoma cruzi*. Cytokine 2017;99:240–248
59 Nagai MY, Dalboni LC, Cardoso TN, et al. Effects of homeopathic phosphorus on encephalitozoon cuniculi-infected macrophages in vitro. Homeopathy 2019;108:188–200
60 Bonamin LV, Carvalho AC, Amaral J, Cardoso TN, Perez EC, Peres GB. Combination of homeopathic potencies, immune response and tumour microenvironment. In: Bonamin LV, Waisse S, eds. Transdisciplinarity and Translationality in High Dilution Research. Signals and Images GIRI Series. Cambridge: Cambridge Scholars Publishing; 2019:211–243
61 Stock-Schröer B. Reporting experiments in homeopathic basic research (REHBaR). Homeopathy 2015;104:333–336
62 Chirumbolo S, Bjørklund G. Homeopathic dilutions, Hahnemann principles, and the solvent issue: must we address ethanol as a "homeopathic" or a "chemical" issue? Homeopathy 2018;107:40–44
63 de Oliveira CC, de Oliveira SM, Godoy LM, Gabardo J, Buchi Dde F. Canova, a Brazilian medical formulation, alters oxidative metabolism of mice macrophages. J Infect 2006;52:420–432
64 Arora S, Aggarwal A, Singla P, Jyoti S, Tandon S. Anti-proliferative effects of homeopathic medicines on human kidney, colon and breast cancer cells. Homeopathy 2013;102:274–282