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ORIGINAL PAPER

Anti-proliferative effects of homeopathic medicines on human kidney, colon and breast cancer cells

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Objective: **Homeopathy is controversial, due to the claims made for very high dilutions. Although several theories are proposed to understand the mechanisms of action, none are scientifically verified. This study aimed to investigate the efficacy of the selected homeopathic medicines in specific *in vitro* cancer models.**

Methods: **We assessed the cytotoxic activity of selected homeopathic medicines in mother tincture (MT), and ultramolecular dilution (30C, 200C, 1M and 10M) against cell lines deriving from tumors of particular organs, *Sarsaparilla* (*Sars*) on ACHN cells (human renal adenocarcinoma), *Ruta graveolens* (*Ruta*) on COLO-205 (human colorectal carcinoma), and *Phytolacca decandra* (*Phyto*) on MCF-7 (human breast carcinoma). *Sars* was also tested against Madin–Darby canine kidney (MDCK) cells (a non-malignant cell line). Cytotoxicity was measured using the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) method, anti-proliferative activity by trypan blue exclusion assay, apoptosis determined by dual staining the cells with ethidium bromide (EB) and acridine orange (AO) dyes.**

Results: **MTs and ultra-diluted preparations of the three homeopathic medicines had highly significant effects in the respective cancer cell lines, producing cytotoxicity and a decrease in cell proliferation. The effects were greatest with the MTs, but in all cases and persisted, although to a lesser degree in the ultra-diluted molecular preparations. *Sars* showed no effect on MDCK cells. In the homeopathic medicine treated cultures, hallmarks of apoptosis were evident including, cell shrinkage, chromatin condensation and DNA fragmentation.**

Conclusion: **This study provides preliminary laboratory evidence indicating the ability of homeopathic medicines as anticancer agents. Further studies of the action of these homeopathic remedies are warranted.** *Homeopathy* (2013) **102**, 274–282.

Keywords: Homeopathy; *Sarsaparilla*; *Ruta graveolens*; *Phytolacca decandra*; Cancer; Cell culture; Anti-proliferative; Apoptosis; Cytotoxicity

Introduction

Exploration of the use of complementary and alternative medicines (CAM) against cancer is gaining importance. Selection of the homeopathic medicine depends upon the individual's symptoms as well as the extent of the disease.[1] The effectiveness and the mechanism of action of ultramolecular preparations are controversial; there is currently limited research that shows homeopathy has effects beyond the placebo effect.

A comprehensive survey carried out by US National Institutes of Health on the use of complementary health practices in United States, showed that 3.9 million adults and 0.91 million children used homeopathy in 2006.[2] Another survey conducted in Tuscany, Italy, reported that 17% cancer patients used CAM therapies after being diagnosed.[3] In 2010, a survey in a pediatric oncology department in

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Germany showed that 45.2% of the total CAM users were exposed to homeopathy and 76.5% of parents used homeopathy for their child's cancer.[4]

Owing to the lack of documentary evidence on various CAM approaches, the US National Cancer Institute (NCI) developed the Best Case Series: CAM practitioners were encouraged to collect and present their results. The NCI also evaluated a cancer treatment protocol developed at the P. Banerji Homeopathic Research Foundation (PBHRF) in Kolkata, India. This protocol uses specific ultra-diluted natural substances to treat patients with various types of cancers.[5,6] Another study showed increase in cell death in brain cancer cells in comparison to lymphocytes on treatment with *Ruta graveolens* (*Ruta*) according to a Banerji protocol.[7] A study of the action of four ultra-diluted remedies (*Carcinosin*, *Phytolacca*, *Conium*, *Thuja*) against two human breast adenocarcinoma cell lines (MCF-7 and MDA-MB-231) and a normal human mammary epithelial cells (HMLE), showed preferential cytotoxic effects against the two breast cancer cell lines, causing cell cycle delay and apoptosis. *Carcinosin* and *Phytolacca* showed activity resembling paclitaxel, a chemotherapy drug used in breast cancer. These findings demonstrated biological activity of these products at ultra-diluted doses.[8]

Homeopathic medicines are said to have no side effects. Homeopathic remedies have been used to ease the side effects of radiotherapy and chemotherapy including stomatitis and skin problems.[9] There are reports of the efficacy of homeopathic medicines in animal models[10]; but limited study of the *in vitro* action of these dynamized preparations.

Cancer is one of the leading causes of death and the incidence continues to rise as the average life expectancy increases. Colon and breast cancer are among the most prevalent forms worldwide.[11] The incidence of kidney cancer has also increased with obesity and diabetes as predisposing factors.[12–14]

This study was conducted to evaluate the effects of homeopathic medicines against kidney, colon and breast cancers. The model systems chosen for the study were well-characterized, malignant cell lines of human origin. The human renal carcinoma ACHN cell line was used for studies pertaining to kidney cancer. Studies on colon cancer were done using the COLO-205 human adenocarcinoma colon cells. For breast cancer, MCF-7, human breast adenocarcinoma cells were used. The selection of the homeopathic medicines was based on review of literature and consultation with the doctors from the Homeopathic College at Chandigarh, India. Preliminary studies were then undertaken in which all the three homeopathic medicines were tested for their cytotoxic effect using the trypan blue viability assay, against each cell line. The homeopathic medicine, showing the highest cell line specific cytotoxicity, was then chosen specifically for further studies against a particular cancer cell line. Since, conventional chemotherapy kills normal as well as the cancer cells, leading to various side effects, we included in our study an immortalized non-malignant kidney cell line, Madin–Darby canine kidney (MDCK), to assess cytotoxic effect of homeopathic medicine *Sarsaparilla* (*Sars*) on a non-malignant kidney cell line.

Materials and methods

Homeopathic remedies

The medicines were procured from Dr Reckeweg & Co., Pekana and Wilmar Schwabe, Germany. The mother tincture (MT), 30C, 200C, 1M and 10M of all the three homeopathic medicines were used against a specific cell line. *Sars*, was tested on a kidney cancer cell line (ACHN) and normal kidney cell line (MDCK), *Ruta*, on a colon cancer cell line (COLO-205) and *Phytolacca decandra* (*Phyto*), against breast cancer (MCF-7).

The batch numbers of the homeopathic remedies obtained were: *Ruta* MT-1102020, *Ruta* 30C-5677IN 353130, *Ruta* 200C-5681IN349190, *Ruta* 1M-0310, *Ruta* 10M-5698IN 342070; *Sars* MT-0210, *Sars* 30C-5828IN344050, *Sars* 200C-5831IN329250, *Sars* 1M-5835IN305163, *Sars* 10M-5839IN307033; *Phyto* MT-0110, *Phyto* 30C-5234IN335200, *Phyto* 200C-5241IN339090, *Phyto* 1M-0110, *Phyto* 10M-5241IN339090.

Since these were prepared in alcohol (90% ethanol), we included a solvent control (SC) in which a final concentration per well of 0.45% of 90% alcohol (i.e., 5 μL/ml) was added instead of the homeopathic medicine. At this concentration of the solvent the cell viability was comparable to control, i.e., >90% for all the three cancer cell lines used in this study as well as the normal cell line (MDCK). Therefore we hoped that the results that would be observed on treatment of the cancer cells would be due to the homeopathic preparation only. The homeopathic medicines were tested for a period of 48 h for cytotoxicity, cell death and proliferation (Table 1).

Cell lines

Three malignant (COLO-205, MCF-7, and ACHN) and the non-malignant MDCK kidney cell lines were used as *in vitro* models for the study. All were obtained from National Center for Cell Science, Pune, India. Dulbecco's modified Eagle medium (DMEM) used for MCF-7 and MDCK, Roswell Park Memorial Institute medium (RPMI) used for COLO-205 and Minimal Essential Media (MEM) used for ACHN were obtained from Sigma–Aldrich, India. The cells were cultured in their respective media supplemented with 1% (v/v) Penicillin–Streptomycin obtained from Gibco and 10% (v/v) FBS obtained from Sigma–Aldrich, maintained at 37°C in a 5% CO_2 incubator. Cells at exponential stage were used for experimentation and medium was changed every 3–4 days.

Cytotoxicity assessment by 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay

The yellow tetrazolium MTT is reduced by metabolically active cells (viable) by the action of dehydrogenase enzymes to generate an insoluble purple formazan in the

Table 1 Overview of the study design

Parameter assessed (performed three times each, in duplicates)	*Cell line and homeopathic medicine*	*Control (untreated cells)*	*SC*	*MT*	*30C*	*200C*	*1M*	*10M*
Cytotoxicity by MTT	ACHN-*Sars*, COLO-205-*Ruta*, MCF-7-*Phyto*, MDCK-*Sars*	X	X	X	X	X	X	X
Fluorescence based analysis of cell death	ACHN-*Sars*, COLO-205-*Ruta*, MCF-7-*Phyto*	X	X	X	X	X	X	X
Anti-proliferative assay by trypan blue exclusion	ACHN-*Sars*, COLO-205-*Ruta*, MCF-7-*Phyto*	X	X	X	X	X	X	X

mitochondria of living cells.[15–18] 10^4 cells/well, 7×10^3 cells/well, 65×10^2 cells/well and 8×10^3 cells/well, of ACHN, COLO-205, MCF-7 and MDCK cells, respectively, were seeded in 96-well plates. Once the cells reached 80% confluency, the respective homeopathic medicine or solvent alone was added as 5 μL/ml in complete growth medium. After 48 h of treatment, 10 μL/well of MTT (5 mg/ml) was added and incubated at 37°C for 4 h. The formazan products were solubilized by 100 μL/well of DMSO. Optical density (O.D) was measured at 570 nm with background wavelength 630 nm using Bio-Rad 96-well reader. The relative % age of cytotoxicity was a comparison between the O.D of the control cells (untreated) versus the cells subjected to various treatments and was calculated as follows:

$$\text{Relative \% cytotoxicity} = 100 - \left\{\frac{(\text{O.D of the treated sample}) \times 100}{(\text{O.D of control sample})}\right\}$$

Fluorescence microscopic analysis of cell death

Staining cells with fluorescent dyes, including acridine orange (AO) and ethidium bromide (EB), are used for evaluating the nuclear morphology of apoptotic cells.[19] AO is a vital dye that stains both live and dead cells; EB stains only cells that have lost their membrane integrity. Briefly, the cells were seeded at approximately 4×10^4 cells/well in a 24-well plate. Once the cells reached 80% confluency, the respective homeopathic medicine or the solvent alone was added as 5 μL/ml in complete growth medium. After 48 h of incubation, both adherent and cells in suspension were collected and centrifuged at 130 g for 5 min. The pellet was resuspended in a solution of 25 μL PBS and 2 μL EB/AO dye (100 μg/ml). Slides were prepared and fluorescence was observed with a Nikon inverted fluorescent microscope at 400× magnification. A green nucleus indicates live cells; bright green nucleus with condensed or fragmented chromatin indicates early apoptotic cells; while late apoptotic cells display condensed and fragmented orange chromatin.

Anti-proliferation assay

To study whether the effect of the homeopathic medicines on the growth of the cancer cell lines was a reversible or irreversible, we modified the trypan blue exclusion method for cell viability to count the number of cell remaining after the medicine had been removed and the cells allowed to proliferate in the presence of complete media.[20] Briefly, 4×10^4 cells/well were seeded in a 24-well plate. Once the cells reached 80% confluency, 5 μL of the respective homeopathic medicine or the solvent alone was added per mL of complete growth medium. After 48 h of incubation, old medium was replaced with complete growth medium without homeopathic medicine or solvent and the cells further incubated for another 24 h or 48 h. To check the effect on cell proliferation after removal of homeopathic medicine, cell viability assay using the vital dye, trypan blue was performed.

For each experiment three plates were run in parallel and the cell count was done follows.

Plate 1: Post 48 h homeopathic medicine addition
Plate 2: 24 h Post medium replacement (total treatment period of 72 h)
Plate 3: 48 h Post medium replacement (total treatment period of 96 h)

Statistical analysis

All the experiments were performed three times; each time in duplicate. The parametric one-way ANOVA test was applied to test the significance of the various data collected during this investigation. The software used was GraphPad InStat.

Results

Several approaches were used for the determination of cell viability/cytotoxicity in response to treatment. These include MTT which takes advantage of enzymes present in the mitochondria of live cells to convert a yellow soluble dye into an insoluble purple colored product. This conversion can be directly related to the number of viable cells present in the sample. By comparing the amount of purple formazan produced by cells treated with an agent against the amount produced by control cells, the effectiveness of the agent in causing death (cytotoxicity) of cells can be deduced.

We observed that on treating the malignant ACHN, COLO-205 and MCF-7 cell lines for a period of 48 h with their respective homeopathic medicines, significant cytotoxicity ($P < 0.001$) was seen at all the potencies with respect to the controls (untreated cells). However, in comparison to all the potencies tested, the MT showed the maximum effect cytotoxicity in all three-cell lines, (Table 2). Treating the ACHN kidney cancer cell line with the MT of *Sars* lead to 82.3% cytototoxicity. *Ruta* showed 66.5% and *Phyto* showed 72.6% cytotoxicity in COLO-205 and MCF-7 cancer cell lines, respectively. The 30C dilutions were associated with 48.6−42.2% cytoxicity, and the effect gradually declined with increasing dilution to 21.8−28.8% with the 10M preparations, however these were still statistically highly significantly different from control. The cytotoxic effect was not attributable to the solvent as the cytotoxicity observed with the SC varied from 2.1 to 3.7%. Treatment of the normal kidney epithelial cell line (MDCK) with various potencies of *Sars* (Table 2d) revealed that the homeopathic medicine spared normal cells: *Sars*-treated MDCK cells showed a maximum cytotoxicity of 22.1%.

We also examined whether the cell death observed could be attributed to apoptosis, since it is thought that cancer cells owe their enhanced lifespan to an evasion of apoptotic pathway. Hallmarks of apoptosis were evident on treatment with different potencies in all the three malignant cell lines. Features characteristic of apoptosis, including blebbing of cell membrane, chromatin condensation and DNA fragmentation were observed in the treated cells using EB/AO double staining (Figures 1−3) indicating that increase in cell death on treatment on different potencies is due to apoptosis.

Table 2 Cytotoxicity assessed by MTT assay in four cell lines treated for 48 h with MT and various potencies of *Sars*, *Ruta* and *Phyto*. a: *Sars* on ACHN. b: *Ruta* on COLO-205. c: *Phyto* on MCF-7. d: *Sars* on MDCK. Values are mean ± SD. All experiments $n = 3$; each time in duplicate. One-way ANOVA was used to compare versus the control. ***$P < 0.001$

Potency	*Percentage cytotoxicity with respect to controls*
a: *Sars*/ACHN	
MT	82.3 ± 9.8***
30C	48.6 ± 3.6***
200C	42.1 ± 7.0***
1M	36.5 ± 3.9***
10M	31.7 ± 4.1***
SC	2.7 ± 0.3
b: *Ruta*/COLO-205	
MT	66.5 ± 5.0***
30C	42.6 ± 6.4***
200C	37.5 ± 9.3***
1M	29.9 ± 7.2***
10M	21.8 ± 4.6***
SC	3.1 ± 0.7
c: *Phyto*/MCF-7	
MT	72.4 ± 0.3***
30C	42.2 ± 2.8***
200C	35.6 ± 6.7***
1M	21.5 ± 3.1***
10M	28.8 ± 3.6***
SC	2.1 ± 0.5
d: *Sars*/MDCK	
MT	22.1 ± 7.6
30C	7.2 ± 2.1
200C	6.1 ± 1.5
1M	5.4 ± 1.9
10M	5.2 ± 2.4
SC	4.2 ± 0.5

Proliferation assay was performed to check the effect on recovery of the cell cycle after replacement of homeopathic medicine containing medium with fresh medium. Cells were treated with the various homeopathic medicines for a period of 48 h and then counted (plate 1) or following the removal of medium and addition of fresh medium (minus the homeopathic medicine) in plate 2 incubated for another 24 h or plate 3 incubated for 48 h and were then counted. A marked effect on the cell proliferation pattern in presence of homeopathic medicine with a tendency to normalize on removal of homeopathic medicine containing medium, was observed, the effect was slowest for *Sars* (Figure 4). The data revealed that compared to the controls, all potencies of the three medicines under study were associated with a significant decrease ($P < 0.05$) in viable cell count, at all the time periods studied. The maximum decrease was seen in the case of MT and 30C for all three homeopathic medicines. For MT, *Sars* showed 15.4 ± 0.65% and 14.5 ± 0.41% viability 24 and 48 h post treatment, respectively. *Ruta* showed 32.4 ± 0.5 and 32.8 ± 0.81% viability after 24 and 48 h post treatment, while *Phyto* showed 39.7 ± 1.75 and 44.2 ± 1.38% viability on removal of homeopathic medicine containing medium after 24 h and 48 h, respectively. Again the effect was greatest with the MT, followed by the 30C and gradual declining with increasing potency, but remaining statistically significant at all dilutions, compared to control.

Discussion

Homeopathic remedies are prepared in sequential steps of dilutions of homeopathic stock (MT). After several steps of serial dilutions, the remedies reach calculated dilutions beyond Avogadro's number, implying a non-molecular action of remedies with specific healing properties. Although there are a number of questions regarding the scientific basis for the efficacy of these homeopathic medicines, researchers are searching for *in vitro* effects of potentized remedies on molecular or cellular systems.[21]

Various studies have been conducted in animal models,[10] showing a decrease in cell viability of the tumors of the respective models. A recent study showing *in vitro* and *in vivo* anticancer properties of *Calcarea carbonica* derivative complex M8 (highly diluted natural product) in a murine melanoma model, demonstrated inhibition of tumor growth and metastasis by decrease in perlecan expression (component of basement matrix and its expression correlates strongly with the expression of several metastatic, angiogenic and invasive factors in tumor cells, particularly melanoma cells) and a direct inhibition of cancer cell adhesion and invasion.[22,23] Another study revealed that on treatment with a complex homeopathic medication (*Calcarea carbonica* CH5) there was an indirect

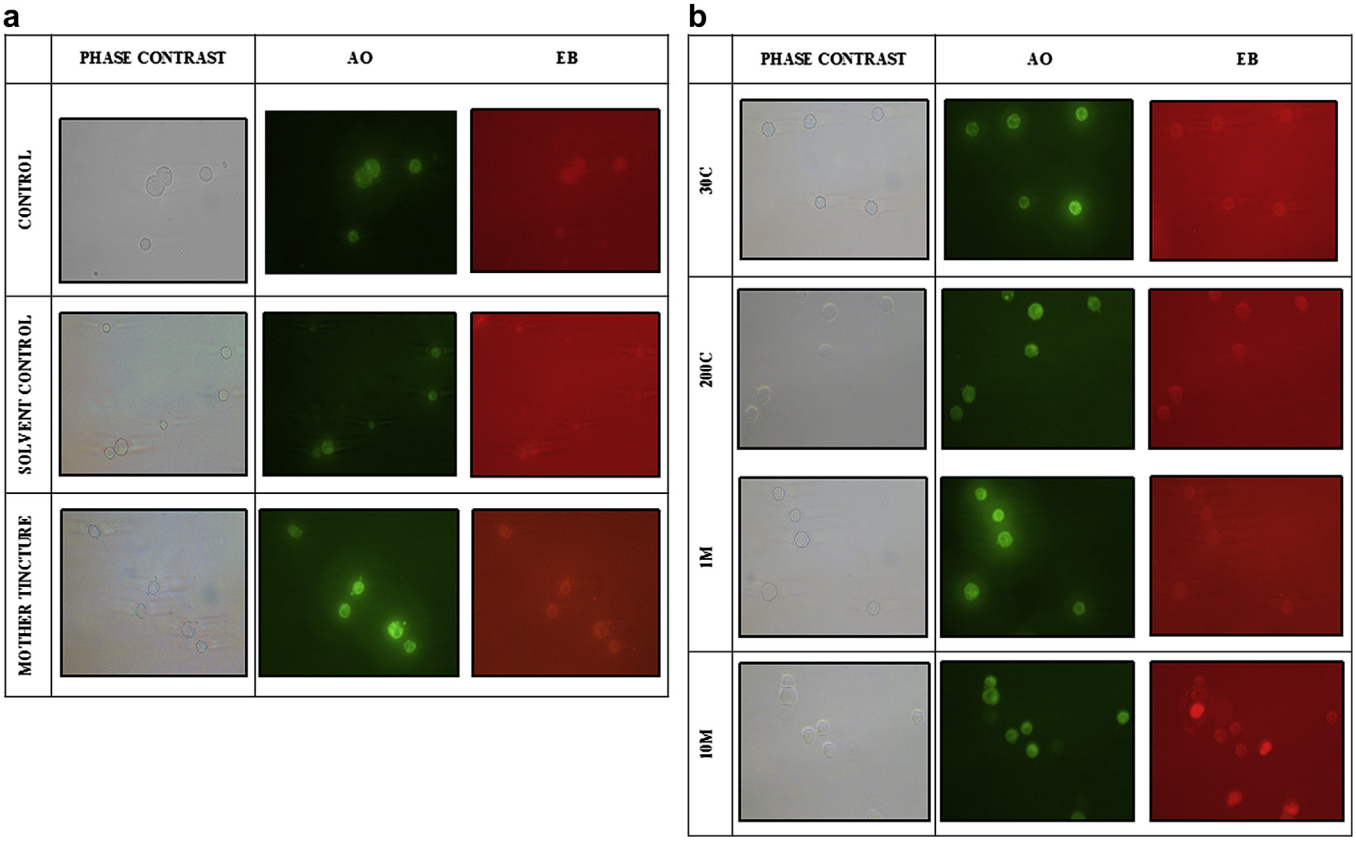

Figure 1 a: Apoptotic changes in ACHN cells treated with different potencies of *Sars* by EB/AO double staining. Cells were stained with EB and AO dye and visualized by fluorescence microscope at 400×. b: Apoptotic changes in ACHN cells treated with different potencies of *Sars* by EB/AO double staining. Cells were stained with EB and AO dye and visualized by fluorescence microscope at 400×.

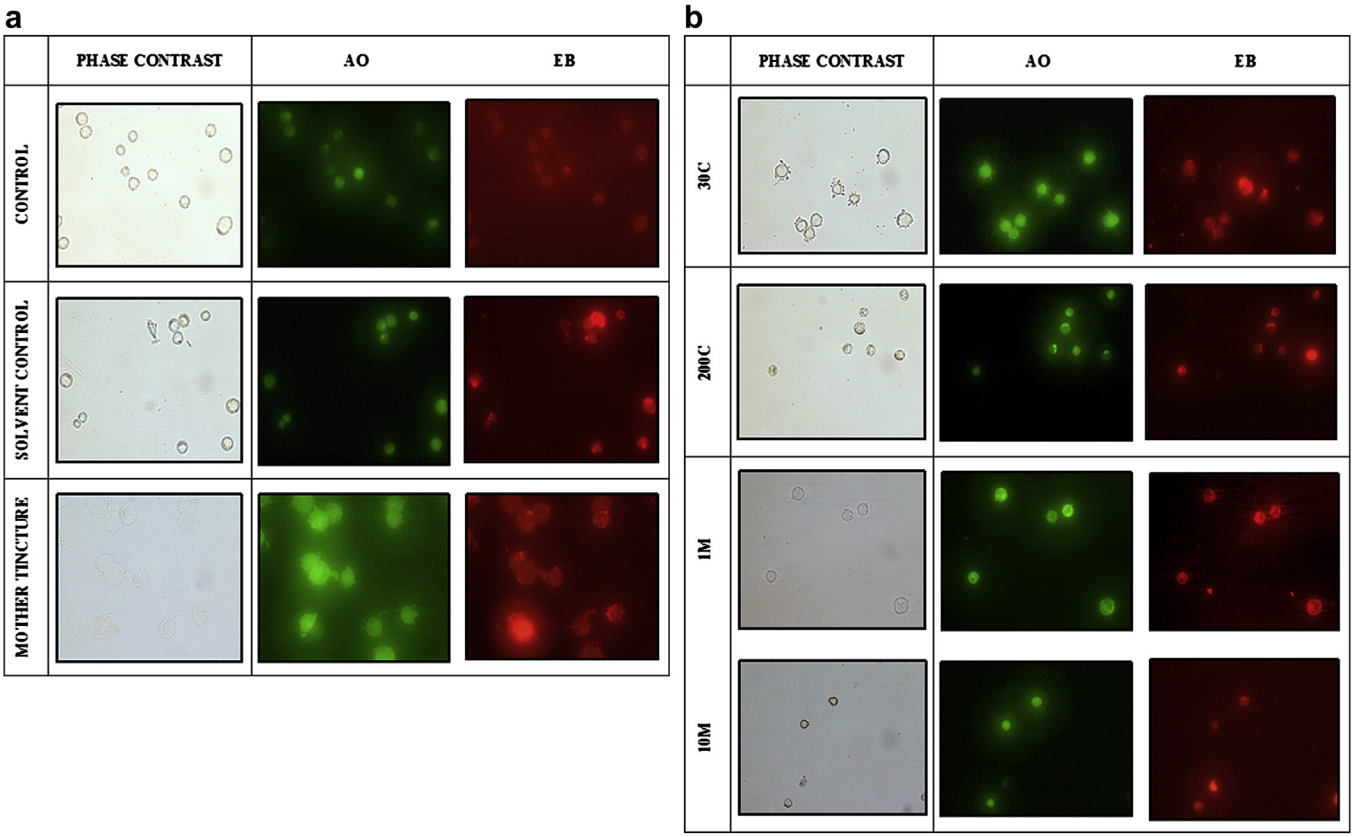

Figure 2 a: Apoptotic changes in COLO-205 cells treated with different potencies of *Ruta* by EB/AO double staining. Cells were stained with EB and AO dye and visualized by fluorescence microscope at 400×. b: Apoptotic changes in COLO-205 cells treated with different potencies of *Ruta* by EB/AO double staining. Cells were stained with EB and AO dye and visualized by fluorescence microscope at 400×.

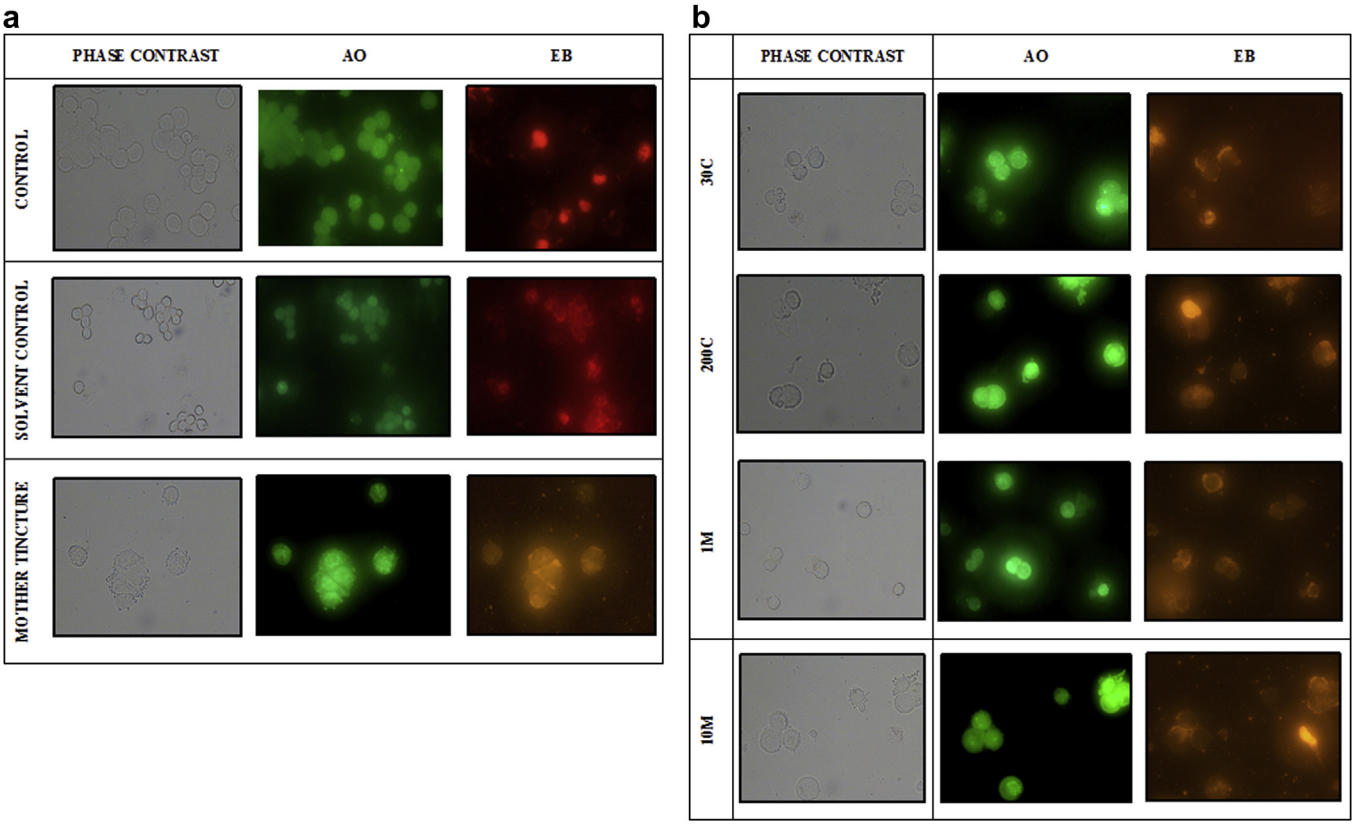

Figure 3 a: Apoptotic changes in MCF-7 cells treated with different potencies of *Phyto* by EB/AO double staining. Cells were stained with EB and AO dye and visualized by fluorescence microscope at 400×. b: Apoptotic changes in MCF-7 cells treated with different potencies of *Phyto* by EB/AO double staining. Cells were stained with EB and AO dye and visualized by fluorescence microscope at 400×.

activation of lymphocytes through their interaction with macrophages, even without direct cell−cell contact. The co-culture of macrophages and lymphocytes in the presence of CH5 promoted immunostimulation of lymphocytes, resulting in enhanced tumoricidal performance against a very aggressive line of melanoma cells. Also, the lymphocytes activated by the treatment destroyed growing cancer cells more effectively than control lymphocytes.[10]

We investigated whether the homeopathic medicines traditionally prescribed to patients with cancer affecting particular organs, at various potencies, elicited a similar response in an isolated model system i.e., specific cancer cell lines without the modulating effect of the immune system. Our study has shown maximal effectiveness of MT and 30C, but with statistically significant effects at all dilutions tested. The persistence of cytotoxic effects far into the ultramolecular range is puzzling, but our observations are not without precedent. Frenkel *et al.* studied the effects of potencies ranging from 3C to 200C on breast cancer. In their study, four different remedies *Carcinosin* 30C, *Conium maculatum* 3C, *Phyto* 200C and *Thuja occidentalis* 30C were used on MCF-7 and MDA-MB-231 cells, the inhibitory effects were higher for the longer period of treatment and at higher doses. *Carcinosin* and *Phytolacca* reduced viability of the MCF-7 cells by 60−75% at a dose of 5 μL/ml and by 70−80% at 10 μL/ml dose after 48 and 72 h treatment, respectively. For the MDA-MB-231 cells, the reductions were 50−65% at a dose of 5 μL/ml and 65−70% at a dose of 10 μL/ml.[8] Sunila *et al.* used MTs as well as some dynamized medicines and showed significant cytotoxicity to cells during short and long-term incubation. They studied the effect of 10 different homeopathic medicines on various cell lines at varying potencies including those of MT, 30C and 200C and in most of the homeopathic remedies tested the MT showed greatest cytotoxicity. In some cases the cytotoxicity induced by 30C was more than 200C.[24] Therefore our results are in agreement with theirs. Guimarães *et al.* attributed the anti tumoricidal effect of homeopathic preparations on their ability to prime the immune system to seek out and destroy cancer cells.[23] It is possible that *in vivo* ultra-diluted higher potencies alter the immune system resulting in enhanced cytotoxicity against cancer cells. But our *in vitro* study was based on homogenous cell populations, with no immune cell component. Biswas *et al.* demonstrated that MT of *Thuja occidentalis* showed maximum cytotoxic effect on A375 cell line.[25]

Cell death can be by necrosis or induction of apoptosis.[26] To confirm whether these potencies are inducing morphological alterations that could be attributed to an apoptotic mechanism. Various potencies of homeopathic remedies traditionally used to treat cancer at particular sites were tested on specific cancer cell lines for 48 h. Untreated control cells showed green fluorescence, due to exclusion of EB but not of AO, while apoptotic cells acquired a bright

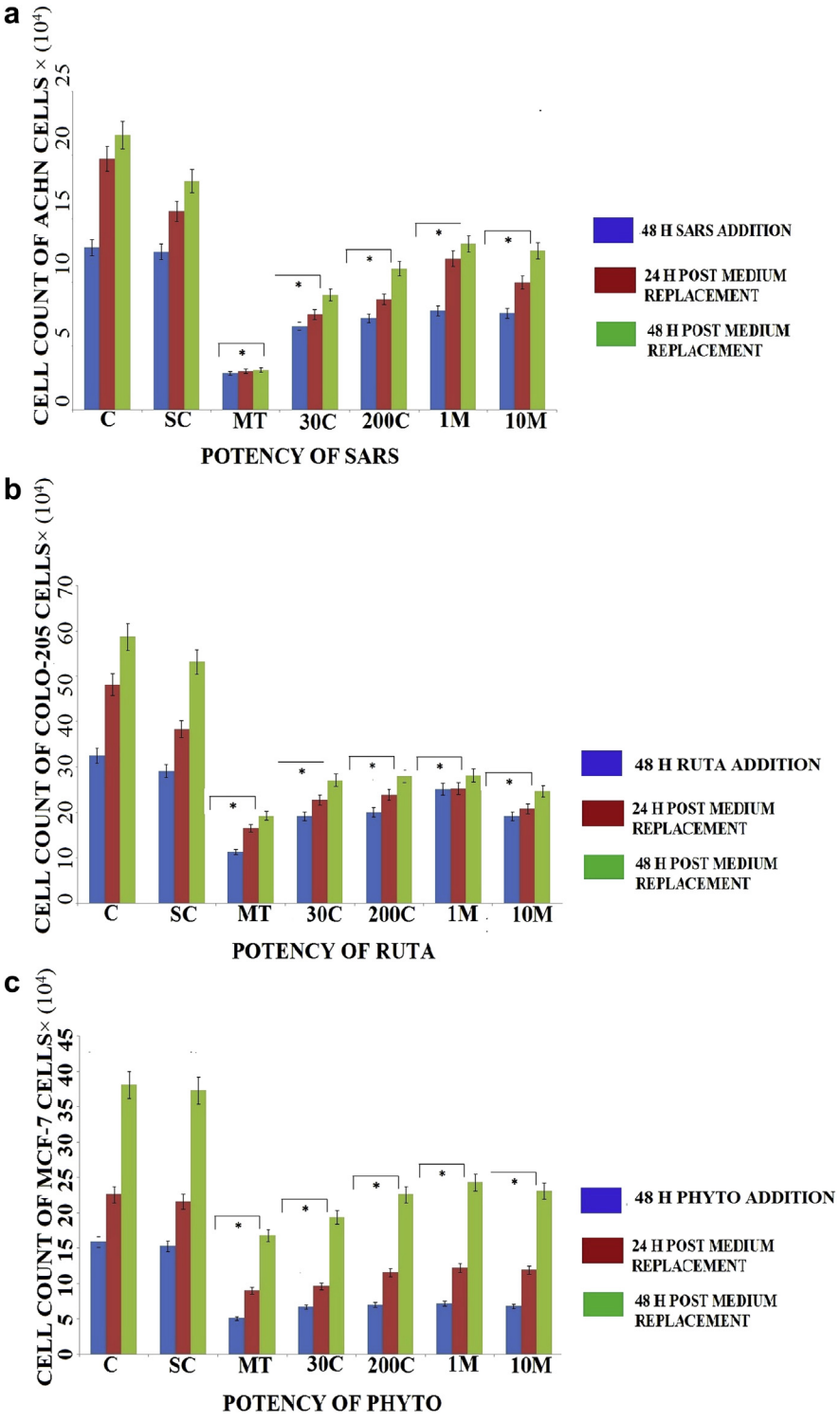

Figure 4 Anti-proliferation assay. Effect of the homeopathic treatments on cell growth as assessed by cell count measurements by trypan blue exclusion assay for a period of 48 h, followed by post medium (minus treatment) replacement for a further 24 or 48 h. (a) *Sars* against ACHN and (b) *Ruta* against COLO-205 (c) *Phyto* against MCF-7. C indicates untreated control cells, SCs. The values are means ± SD, obtained from $n = 3$, independent experiments performed in duplicate. One-way ANOVA was used to compare different potencies of the medicines, on respective cell lines versus their time specific control. $^*P < 0.05$.

green nucleus (showing condensation of chromatin), dense green areas and evident membrane blebbing. In certain cells the condensed nuclei were stained with orange dye due to uptake of EB, indicating compromised membrane integrity which usually happens at the later stages of apoptosis.[27,28]

Analyzing the effects on cell growth inhibition and/or cell death has been an important component of much biological research, especially in cancer treatment development. Accurately measuring the effects of treatments upon cells, *in vitro* can be a challenging task. Proliferation assays were performed for all the three cell lines for the

respective homeopathic medicines. Cell count was performed post 24 h and 48 h medium replacement. Results of the proliferation assay have thrown some interesting light on the mechanism of action of these homeopathic medicines. In the case of *Phyto* and *Ruta* there is a significant effect on the viability of cells exposed to them. On removal of the homeopathic medicines the cells again resume their proliferation cycle although at a slower rate. The inference drawn from such a comparison indicated that the homeopathic medicines affect the cancer cells by increasing the doubling time of the treated cells as compared to untreated cells, this occurs when the cell cycle is disrupted.[17] *Sars* however seems to induce cytotoxicity and its effect seems to continue even after it was removed from the medium. This indicates that effect on the cell cycle is irreversible. A number of chemotherapeutic agents act in a similar manner by irreversibly altering the cell cycle and pushing the cells from the G1 to the G0 stage. Finally, the cytotoxic effects on renal carcinoma cells (ACHN) were compared with cells derived from normal renal epithelium (MDCK) showing no significant cytotoxic effect of *Sars* on MDCK.

Conclusion

Our data indicates the potential as anticancer agents of these homeopathic medicines which can be attributed in part to its inhibition of proliferation and apoptosis. *Sars*, owing to its irreversible effect on proliferation and sparing of normal cells is particularly interesting.

The homeopathic medicines tested in this study *Sars*, *Phyto* and *Ruta* demonstrated both cytotoxic as well as anti-proliferative activity. The effect of *Phyto* and *Ruta* reduced after they were removed from the medium, but they present a possibility of slowing down the proliferation rate of the cancer cells.

This study provides preliminary *in vitro* evidence indicating the potential of homeopathic medicines in the treatment of cancer. Further research should be conducted. Understanding the mechanism of action of ultramolecular dilutions presents a major challenge.

Author disclosure statement

No competing financial interests exist.

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