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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