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• **Research Article**

Psorinum 6× triggers apoptosis signals in human lung cancer cells

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ABSTRACT

OBJECTIVE: To provide *in vitro* evidence of Psorinum treatment against cancer cells in a controlled study.

METHODS: Effects of homeopathic Psorinum 6× on cell viability were initially determined in several cancer cell lines, including A549, HepG2 and MCF-7, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and an ethanol 6× control. The cell line that exhibited highest inhibition was selected and used in the following experiments. A range of Psorinum 6× doses was used to explore treatment effects on cell cycle arrest, cell death (apoptosis), generation of reactive oxygen species (ROS) and change in mitochondrial membrane potential (MMP) using flow cytometry and fluorescence microscopy, respectively. Expression of several signal proteins related to apoptosis and cell survival were quantified with Western blotting and confocal microscopy. Further, circular dichroism (CD) spectroscopy was used to determine possible drug-DNA interactions, as well as the induction of conformational changes.

RESULTS: Treatment of cancer cell lines with Psorinum showed greater anticancer effects in A549 cells than in others. In A549 cells Psorinum treatment inhibited cell proliferation at 24 h after treatment, and arrested cell cycle at sub-G_1 stage. It also induced ROS generation, MMP depolarization, morphological changes and DNA damage, as well as externalization of phosphatidyl serine. Further, increases in p53 expression, Bax expression, cytochrome *c* release, along with reduction of Bcl-2 level and caspase-3 activation were observed after Psorinum 6× treatment, which eventually drove A549 cells towards the mitochondria-mediated caspase-3-dependent pathway. CD spectroscopy revealed direct interaction of Psorinum with DNA, using calf thymus-DNA as target.

CONCLUSION: Psorinum 6× triggered apoptosis in A549 cells via both up- and down-regulations of relevant signal proteins, including p53, caspase-3, Bax and Bcl-2.

Keywords: homeopathy; Psorinum therapy; lung neoplasms; reactive oxygen species; anticancer potential; apoptosis; drug-DNA interaction

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1 Introduction

Great efforts have been made to successfully combat various types of cancer including improved surgical tools, radiotherapy and chemotherapy. However, such interventions, due to financial limitations, are not available to huge populations, particularly in the developing world[1–6]. Further, the number of cancer cases

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has increased so much so that many cancer patients are unable to obtain mainstream cancer treatments because of inadequate medical infrastructure, in addition to financial constraints[5,6]. Additionally, the prognosis of some forms of cancer, like cancers of liver, gall bladder, pancreas, and stomach, is still poor[7]. Elderly cancer patients may not tolerate conventional cancer treatments because of old age-related problems[8,9], including lack of strength to cope up with chemotherapy and/or radiotherapy. Therefore, in recent years, there has been a serious search for efficient complementary and alternative medicines (CAMs) which could render additional benefits, particularly when used in combination with the mainstream Western medicines[10,11]. Psorinum therapy is one CAM practice, whose promising results have been advertised by a group of Indian scientists and clinicians[12,13]. However, these studies are based on single-armed experimental design, without any control, weakening their conclusions. Thus they do not provide the convincing evidence needed to recommend the safe use of Psorinum treatment in human subjects. In fact, in the homeopathic regimen, it has been suggested that quite a few remedies have considerable anticancer effects[14]. As a result, alternative cancer treatments have gained considerable importance in oncology throughout the world. Among several homeopathic treatment protocols now being used in India (*e.g.*, Banerjee protocol[15] and Sankaran protocol[16]), with varying degree of success, Psorinum therapy[12,13,17,18] is believed to treat several forms of cancer successfully, enabling the patients to survive for several years with improved quality of life.

The drug Psorinum 6× ("×" stands for decimal potency of homeopathy), is derived from an alcoholic extract of slough, and pus cells from scabies. The 6× potency of Psorinum is claimed to activate different immune effector cells (*e.g.*, T cells, and accessory cells like macrophages, dendritic cells, and natural killer cells) which can trigger a complex antitumor immune response[18]. Daily oral administration of Psorinum 6× to albino rats, at doses up to 0.5 mL/kg body weight, for 2 weeks, resulted in no adverse side effects[19]. Published retrospective and prospective accounts claim considerable efficacy of Psorinum therapy in treating patients with various malignancies, namely, stomach, gall bladder, pancreatic and liver cancers, without showing any significant side effects[20]. Unless a controlled *in vitro* study can provide decisive proof of its anticancer potential, its human use will be restricted.

Therefore the present *in vitro* controlled study was undertaken, using adenocarcinoma cells A549, in order to gain preliminary knowledge about its efficacy and probable molecular mechanism of action. Our focus was particularly on the signaling pathway, as cervical cancer cells HepG2 and breast cancer MCF-7 cells were also found to show cytotoxicity induced by Psorinum 6× administration, in preliminary studies.

2 Materials and methods

2.1 Chemicals and reagents

Dulbecco's modified Eagle medium (DMEM), penicillin, streptomycin and neomycin (PSN) were purchased from HiMedia (India). Fetal bovine serum (FBS), trypsin and ethylene di-amine tetra-acetic acid (EDTA) were obtained from Gibco BRL (Grand Island, NY, USA). Tissue culture plastic wares were procured from Tarson (India). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), dichloro-dihydro-fluorescein diacetate (H_2DCFDA), 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) and rhodamine 123 were purchased from Sigma (USA). Annexin V-fluorescein isothiocyanate (FITC) and primary antibodies were obtained from Santa Cruz Biotechnology Inc., (USA). Secondary antibodies were purchased from Sigma.

2.2 Source of homeopathic Psorinum 6×

The Psorinum nosode is made from fluid discharged by blisters in the skin caused by a scabies (contagious mites) infestation[17]. It is considered to be one of the most effective remedies for many skin problems that do not respond to other treatments[17]. Homeopathic potencies of Psorinum 6× are made by trituration (grinding thoroughly) of the secretions with lactose, or in 70% ethanol in serial dilutions on a decimal scale (*i.e.*, diluted in the scale of 10 at each "potentization" step of dilution). The nosode Psorinum 6× was kindly provided by Hahnemann Publishing Company Private Limited, BB Ganguly Street, Kolkata.

2.3 Cell culture

MCF-7, HepG2, A549 and WRL-68 cells were collected from National Centre for Cell Science (NCCS), India. MCF-7, HepG2 and A549 cells originated from breast, liver and lung cancer, respectively. WRL68 cells were procured from NCCS Pune as certified derivatives of normal liver hepatocytes. Earlier authors have also used WRL68 as controls for normal liver hepatocytes in *in vitro*[21,22] experiments, as the cells could synthesize all liver marker enzymes. The cells were maintained in a humidified incubator (ESCO, Singapore) at 37 °C, with ambient oxygen and 5% carbon dioxide. Cells were cultured in DMEM with 10% heat-inactivated FBS and 1% PSN. Cells harvested with 0.025% trypsin-EDTA in phosphate-buffered saline (PBS) were plated at required cell numbers and allowed to adhere for required time before treatment.

2.4 Cell viability assay

MCF-7, HepG2, A549 and WRL-68 cells were dispensed into 96-well flat bottom micro-titer plates at a density of 1×10^3 cells per well. Different cells were