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Review Article

HERBAL NANOSUSPENSION: *IN VITRO* CANCER STUDY AGAINST DIFFERENT CELL LINES

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ABSTRACT

Herbal formulations marketed in India for many years providing its therapeutic benefits in health problems. Medicinal plants or their phytoconstituents are less toxic and free from side effects than synthetic drugs. However, formulation aspects of these phytoconstituents are limited due to its low solubility. Nanotechnology is a promising technique to increase the solubility of herbal drugs. This will lead to a subsequent reduction in drug dose. Nanoformulations such as nanosuspension increase the solubility of poorly soluble drugs and also have good targeting effects on different cells. The efficacy of herbal nanosuspensions evaluated using different cell lines. Here, hepatocellular carcinoma cell line (SMMC-7721), human prostate cancer cell line, human myelogenous leukemia cell line, human epithelial cell line, human breast cancer cell lines (4T1, MCF-7, and MDA-MB-453), carcinomic human alveolar basal epithelial cell line (A549), human umbilical vein endothelial cell line, human colorectal adenocarcinoma cell line (HT-29), and human epithelial carcinoma cell line (HeLa) are used in the evaluation procedures. *In vitro* assays help in the determination of the dose range of drugs for the activity. The present review highlights the *in vitro* cancer studies of herbal nanosuspensions using different cell lines.

Keywords: Nanosuspension, Herbal, *In vitro* cell lines.

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INTRODUCTION

Herbal medicines are used all over the world due to their low toxicity and reduced side effects than synthetic drugs [1]. The greater part of the active constituents of herbal extract is ineffective to pass lipid membranes due to their high molecular size or low solubility. Nanosuspension technology is introduced as a result of many efforts made by formulation scientists and emerged as a potent candidate for the delivery of the poorly soluble drugs in a more efficient manner. Hence, herbal extract in the form of nanosuspension may be beneficial for the treatment of various diseases [2].

In vitro bioassays are important in the evaluation of these herbal nanosuspensions with possible pharmacological effects [3]. Cell-based bioassays are used to find the pharmacological activity of formulations, and for this purpose, it requires identification of a responsive cell line. A cell line can proliferate indefinitely in an appropriate fresh medium and space. These have a major role in studying physiological, pathophysiological, and differentiation process of exact cells [4]. The cell lines possess many characteristics of normal cells, so these are used in the studies to determine whether medicinal plants have defined pharmacological activities [5].

TYPES OF CELL LINES

Finite cell lines

The cell in culture divides only a limited number of times before their growth rate decreases, and finally, they will die. The cell lines with limited culture life spans are known as finite cell lines. The cells normally divide 20–100 times before destruction. The human cells divide 50–100 times.

Continuous cell lines

A few cells in culture may get different morphology and get modified. Such types of cells are capable of growing faster and resulting in an independent culture. The progeny obtained from these modified cells have an unlimited life. They are known as continuous cell lines. These are transformed, immortal, and tumorigenic.

SELECTION OF CELL LINE

Species

Species differences are important while extrapolating the data to humans.

Finite or continues cell lines

Cultures with continuous cell lines are preferred due to their high yield.

Normal or transformed cells

The transformed cells are preferred because they are immortalized and grow quickly.

Availability

It may be produced in the laboratory.

Stability

The stability of a cell line related to cloning and storage.

Growth characteristics

- Population doubling time
- Ability to grow in suspension
- Saturation density
- Cloning efficiency [6].

IN VITRO CANCER CELL LINE STUDY

In vitro evaluation of curcumin nanosuspension against HeLa cell lines

Curcumin is the active ingredient present in the plant turmeric (Curcuma longa) [7], and it has a wide range of pharmacological activities including anti-cancer, anticoagulant, antithrombotics, antioxidation, and antiproliferation[8]. Curcumin used to treat the regression of psoriasis, pre-malignant lesions of the bladder, also the treatment of many cancers such as the cervix, soft palate, stomach, colorectal, and skin cancers [9-13]. Curcumin can be considered as a prospective drug due to its high safety and low cost [14].

HeLa cell lines are the most commonly used human immortal cell lines [15]; originally, it was reported from a female African-American

individual in 1952 as cervical epithelial carcinoma, consequent analysis of tumor pathology, and clinical phenotype identified the first tumor as an uncommon adenocarcinoma [16]. HeLa has a powerful adaptation to culture conditions because it was the first cell line widely cultured [17]. It is epithelial in appearance grows fast with a doubling time of 24 h and shows a scarcity of contact inhibition [18]. These cells can proliferate rapidly compared to other cancer cells [19]. The stable growth of HeLa enabled the development of the polio vaccine [20], and also it is used by scientists in different types of investigations such as disease research, gene mapping, effects of toxic substances in organisms, and radiation on humans [21].

The anticancer activity of curcumin nanosuspension was assessed by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. The HeLa cells were exposed to curcumin nanosuspension and free curcumin solution at different concentrations (5, 10, 20, 40, 80, 160 μg/ml) for 3 days. Empty excipients as negative control were added at equivalent concentrations as that within the tested curcumin nanosuspension. The curcumin nanosuspension and free curcumin solution inhibited the growth rate of tumor cells in a dose-dependent manner. This study suggested that curcumin nanosuspension shows higher anticancer activity than free curcumin at the same concentration due to the presence of nano-sized particles [22].

***In vitro* evaluation of silybin nanosuspension against prostate cancer cell lines (PC-3) and human epithelial cell line (Caco-2) cell lines**

Silybin is a chemical constituent isolated from milk thistle plant (*Silybum marianum*) and used as a therapeutic agent for a variety of diseases, including human colon cancer, liver disease, and prostate cancer. It also suppresses the cell growth and DNA synthesis, and it is chemopreventive targeting inhibit the invasive process in prostate cancer [23-25]. This herb can be used in beta-thalassemia patients due to its immunomodulatory, iron-chelating, and antioxidant properties [26,27].

PC3 cell lines are human prostatic adenocarcinoma cell line that is used in prostatic adenocarcinoma research and drug development. These were isolated from bone metastasis and grew as an adherent layer [28]. It helps to determine biochemical changes in advanced prostate cancer cells and assessing their response to chemotherapeutic agents [29]. Other PCs-3 such as DU145 and LNCaP have low metastatic potential compared to PC-3 cell lines [30]. Protein expression of PC-3 cells may be a characteristic of small cell neuroendocrine carcinoma [31]. For the generation of subcutaneous tumor xenografts in mice, these cell lines can act as a good tool. Hence, it helps to investigate the tumor environment and therapeutic drug functionality [32].

Antitumor effect of prepared silybin nanosuspension and free silybin was done against the PC-3 cell line in an *in vitro* model and evaluated at different concentrations (0.2, 1, 2, 10, 20, and 100 μg/ml) for 12, 24, 3, and 48 h by MTT reduction assay. The inhibition rate of silybin nanosuspension to PC-3 cells increased with the concentration and time. This study suggested that silybin nanosuspension shows a higher inhibition rate than free silybin at the same concentration [33].

Caco-2 cell line originates from the human colon adenocarcinoma tissue, and it forms a monolayer of polarized enterocytes through spontaneous cellular differentiation. Caco-2 cells are shown to explicit many enzymes and display all of the four different transport routes of the intestinal epithelium [34]. Furthermore, the Caco-2 monolayer is a good *in vitro* model of human small intestine mucosa[35].

The anticancer activity of silybin nanosuspension against the Caco-2 cell line was determined by MTT assay. It was performed to measure the cellular viability. For the study, cell lines were incubated with silybin formulations for 8 h and 48 h at a concentration range of 10–500 $\mu g/ml^{-1}$. When the concentration of silybin was below 125 $\mu g/ml^{-1}$, there was no significant difference between the experimental group and the control group. Hence, this study was stated that nanosuspensions were related to no cytotoxic below the concentration of 125 $\mu g/ml^{-1}$ [36].

***In vitro* evaluation of oridonin nanosuspension against human myelogenous leukemia cell line (K562) and SMMC-7721 cell lines**

Oridonin is a natural ent-kaurane diterpenoid extracted from the various plants such as Isodon trichocarpus, Isodon japonicas, and Isodan shikokianus [37-39]. It has various pharmacological effects including anti-inflammation, anti-bacterial, and anti-tumor activity. Mainly, it used to treat human cancers, including prostate cancer, breast cancer, and lung cancer [40-42].

K562 cell lines are myelogenous leukemia cell lines that produce less clumping in culture due to the downregulation of surface adhesion molecules by the fusion gene [43]. The cells are non-adherent and round; also, it shows a proteomic resemblance to undifferentiated granulocytes [44] and erythrocytes [45]. They easily destroyed by natural killer cells [46] because they lack the major histocompatibility complex required to inhibit natural killer cell activity [47].

The *in-vitro* anticancer activity of oridonin nanosuspension against K562 cell was determined by MTT assay at different concentrations of oridonin nanosuspension and free oridonin solution (5, 10, 20, 30, and 40 μmol/l). The incubation time was 12, 18, 24, and 36 h. The anticancer activity of oridonin nanosuspension increases with the concentration and incubation time. This study suggested that the oridonin nanosuspension shows more anticancer activity than a free oridonin solution at the same concentration. Moreover, this enhanced effect due to the presence of nanoparticles [48].

The SMMC-7721 cell line was derived in 1977 from hepatocellular carcinoma patients. This cell line expresses two receptors, that is, epidermal growth factor receptor and vascular endothelial growth factor receptor. It is an useful tool in liver cancer research [49].

MTT assay was used to determine the *in vitro* anticancer activity of the oridonin nanosuspension compared to the free oridonin solution against the SMMC-7721 cell line. The different concentrations (5, 10, 20, 30, and 40 μmol/l) of oridonin nanosuspension and the free oridonin solution used for the cell line study. The incubation period was 12, 24, 36, and 48 h. Both nanosuspension and free solution inhibited the growth of SMMC-7721 cells in a time-dose-dependent manner. This study suggested that at the same concentration, oridonin nanosuspension showed a higher inhibition rate against SMMC-7721 cell line than a free oridonin solution. It may be due to the presence of a high concentration of free oridonin or uptake of nanoparticles or the combination of both [50].

***In vitro* evaluation of gambogenic acid nanosuspension against hepatocellular carcinoma cell line (HepG2) cell line**

Gamboge is a herbal medicine derived from *Garcinia hanburyi* tree, and it shows pharmacological activities such as detoxification, anti-inflammatory, and parasiticide effects. The main active constituent present in the plant includes gambogenic acid and gamboge. Gambogenic acid has a broad-spectrum anti-tumor effect in different cancer cell lines [51,52].

HepG2 is a human liver cancer cell line isolated from the liver tissues. This immortal cell line used in drug metabolism and hepatotoxicity studies [53,54]. These cells are epithelial in morphology, and various proteins such as albumin, fibrinogen, transferrin, and plasminogen are secreted from these HepG2 cell lines [55]. At suitable culture conditions, HepG2 cell lines show robust morphological and functional differentiation [56]. These are good *in vitro* model system for the study of intracellular trafficking, dynamics of bile canalicular, sinusoidal membrane proteins, and lipids in human hepatocytes [57].

The anti-tumor activity of gambogenic acid nanosuspension on the HepG2 cell line was determined by MTT assay. The HepG2 cell line

was exposed to different concentrations (0.5, 1, 2, 4, 8, and 16 μM) of gambogenic acid nanosuspension and free gambogenic acid solution for 48 h. The cytotoxicity of the gambogenic acid solution and gambogenic acid nanosuspension depends on the dose and concentration rate. This study suggested that the gambogenic acid nanosuspension has significant cytotoxicity to HepG2 cells than a gambogenic acid solution at an equivalent dose and it may be due to uptake of nanoparticles [58].

***In vitro* evaluation of Genkwanin nanosuspension against 4T1, MCF-7, MDA-MB-453, A549, and human umbilical vein endothelial cell line (HUVEC) cell lines**

Genkwanin is a flavonoid isolated from Genkwa flos, Rosmarius officinalis [59], and the leaves of *Cistus laurifolius* [60]. It is known as a representative marker for quality control of traditional Chinese prescriptions [61]. It has various pharmacological effects, including antitussive, expectorant, anti-inflammatory [62], anti-bacterial [63], anti-plasmodial [64], radical scavenging [65], chemoprotective [66], and inhibiting 17β-hydroxysteroid dehydrogenase type 1 activities [67].

4T1 is a breast cancer cell line isolated from the mammary gland tissue of the mouse. These epithelial cells are resistant to 6-thioguanine [68]. It is mainly used to study breast cancer metastasis [69], and in live tissues, it is highly aggressive [70].

The MCF-7 is a breast cancer cell line isolated from Caucasian women [71,72] and acronym of Michigan cancer foundation-7 [73]. This noninvasive cell line represents a model of early-stage disease [74].

MDA-MB-453 cell line is a human breast cancer cell line and it has an active glycerol3-phosphate shuttle [75].

A549 cells are human epithelial cell lines developed in 1972 [76]. These types of cell lines are human alveolar basal epithelial cells [77]. These cell lines used for the study of lung cancer and also used for developing a drug against lung cancer [78,79]. It is responsible for the diffusion of water and electrolytes across alveoli. It is widely used as a type 2 pulmonary epithelial cell model for drug metabolism [80]. It is employed in viral research and also been studied for cell line response to tuberculosis [81,82].

HUVEC cell lines are derived from human umbilical vein endothelial cells in the 1970s and used as a laboratory model system for the study of the function and pathology of endothelial cells [83,84].

Anticancer activity of Genkwanin nanosuspension and Genkwanin solution against 4T1, MCF-7, MDA-MB-453, A459, and HUVEC cell lines assessed using MTT assay. Various concentrations (0.5, 1, 2, 5, 10, 20, 50, 100 μg/ml) of Genkwanin nanosuspension and Genkwanin solution were used for the study. The incubation time was 48 h. Both Genkwanin nanosuspension and Genkwanin solution inhibited cell proliferation in a dose-dependent way. This study suggested that Genkwanin nanosuspension exhibited higher cytotoxicity than the Genkwanin solution against all studied cell lines [85].

***In vitro* evaluation of puerarin nanosuspension against HT-29 cell line**

Puerarin is an isoflavone [86] isolated from the root of the Pueraria lobata [87]. It have many beneficial effects on liver disease [88,89],cardiovascular [90,91], neurological [92,93], anti-platelet aggregation [94], and hyperglycemic disorders [95]. Furthermore, it exhibits protective actions against fever, inflammation, hyperlipidemia, osteonecrosis, and oxidative damage [96].

HT-29 cell lines are a colon cancer cell line utilized in cancer research [97]. These cells are useful for somatic cell research due to their ability to differentiate and simulate real colon tissue *in vitro* [98]. HT-29 cells can proliferate in cell culture with a doubling time of around 4 days without using the growth factors. The doubling time is often reduced to 1 day with added fetal bovine serum [99]. The HT-29 cells have shown induced differentiation [100], with galactose-mediated differentiation, also results in the strengthening of adherens junctions [101].

The antitumor activity of puerarin nanosuspension was evaluated by MTT assay. Various concentrations (1.25, 2.5, 12.5, and 25 $\mu g/ml^{-1}$) of puerarin nanosuspension and puerarin solution incubated for 8, 16, and 24 h. Both the puerarin nanosuspension and puerarin solution inhibited the cell proliferation of HT-29 in a concentration time-dependent manner. This study suggested that compared with puerarin free solution, nanosuspension of puerarin showed a significant increase in the cell inhibition rates under the same concentrations. The inhibitory effects increased with concentration and incubation time [102].

CONCLUSION

Herbal drugs have low side effects than synthetic drugs and also if it is formulated as nanosuspensions that it increases the solubility of drugs than conventional dosage forms. For the *in vitro* evaluation of herbal nanosuspensions, cell lines are mostly preferred. Because it offers easy, inexpensive, and stable platforms for the study. Here, HepG2, PC-3, K562, Caco-2, SMMC-7721,4T1, MCF-7, MDA-MB-453, A549, HUVEC, HT-29, and HeLa cell lines are used in the evaluation of different herbal nanosuspensions. Each cell line is derived from various organs and sources. MTT assays are the most commonly used method in the cell line studies to estimate the number of viable cells in multi-well plates. Cell metabolic activity can be determined using this assay method. Hence, cell lines can be used as an *in vitro* model for evaluation of herbal nanosuspensions.

AUTHORS' CONTRIBUTIONS

The authors declare that this work was done by the authors named in this article.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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