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Original Article

ANTIPROLIFERATIVE EFFECT OF FLOWER EXTRACTS OF SPILANTHES PANICULATA ON HEPATIC CARCINOMA CELLS

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ABSTRACT

Objective: The development and evaluation of new antiproliferative drugs obtained from natural resources has gained its importance because of their less cytotoxic properties. There is no such report regarding antiproliferative effect of *Spilanthes paniculata Linn*. flower. Therefore, the present study was undertaken to evaluate the antiproliferative effect of *Spilanthes paniculata Linn*. flower.

Methods: In order to achieve this goal, the dried flowers were extracted in petroleum ether, ethyl acetate and ethanol. All these three extracts of varying concentrations were subjected to further evaluation of antiproliferative action on human hepatoma cell line (Huh-7 cells). In order to understand the mechanism of antiproliferative effect of these three extracts, various studies like caspase-3 enzyme assay, DNA transillumination assay and receptor tyrosine kinase profiling were performed. Separately, we estimated total flavonoid and phenolic contents and *in vitro* free radical scavenging properties of these extracts.

Results: The results indicated that both ethyl acetate and ethanol extracts possessed antiproliferative effect on Huh-7 cells because of their induction of caspase-3 enzymes and inhibition of phosphorylation of various tyrosine kinases. It was observed that during the transillumination assay of the ethyl acetate and ethanol extracts, DNA of Huh-7 cells were also degraded. It was also found that these two extracts possessed potent antiproliferative effect on Huh-7 cells due to the presence of rich amount of phenols and flavonoids.

Conclusion: Based on our data, both ethyl acetate and ethanol extracts might be beneficial for the future development of antiproliferative therapeutics in drug design perspective.

Keywords: *Spilanthes paniculata* flowers, Antiproliferative effect, Huh-7 cells, Caspase-3, DNA transillumination, Tyrosine kinase.

INTRODUCTION

From the ancient periods, human beings have believed on natural products, which are the common source of therapeutics. Egypt, China and India are the common countries where natural products have been used in healthcare system [1]. World Health Organization published a survey report on primary health care system where they explained about 80% of world population has faith on traditional medicines [2].Currently, 119 chemicals obtained from 90 plant species are considered as important therapeutics in different countries [3].

Spilanthes paniculata Linn. (SP) belongs to the family Asteraceae,also known as toothache plant, akalkada (in Sanskrit) and akarkara (in Hindi). The traditional uses of this plant are to relief toothache, rheumatic fever. Leaves are mainly used against various skin diseases whereas the extract of root is used as purgative [4]. The active constituent of SP is N-isobutylamide including spilanthol which is mainly responsible for above mentioned pharmacological actions [5]. Thomas (2011) observed that the different fractions of flower head of SP were active against gram positive and gram negative bacteria [6]. Ali et al., (2012) evaluated the hepatoprotective effect of SP flower on paracetamol induced hepatotoxicity due to presence of rich sources of flavonoid and phenolic contents [7].

Thus, taking into consideration of above mentioned observations, the present study was conducted to evaluate the antiproliferative effect of SP flower on hepatic carcinoma cells (Huh-7 cells) [8,9]. In order to achieve the desired goal, extraction of SP dried flowers (shade drying) was done by using petroleum ether (60-80°C, PE), ethyl acetate (EA) and ethanol (ET). All these extracts were subjected to further screen for antiproliferative action on Huh-7 cells at different concentrations from 0.5 to 100 µg/ml, followed by apoptotic activity induced by caspase-3 enzymes. Receptor tyrosine kinase (RTK) profiling and DNA transillumination assay were also performed to explain the actual mechanism of antiproliferative effects of these extracts. Separately, we also estimated total phenolic and flavonoid contents and free radical scavenging assays of those extracts. We hypothesized for the first time that both EA and ET extract of SP flower had good antiproliferative effect on Huh-7 cells.

MATERIALS AND METHODS

Materials

Dulbecco's modified Eagle's medium (DMEM) and caspase-3 enzyme assay kit were purchased from Invitrogen Bio Services Pvt. Ltd., India. Petroleum ether (PE), ethyl acetate (EA), and ethanol (ET) were purchased from Merck Pvt. Ltd., India. Dimethyl sulfoxide (DMSO, ACS grade), ethylenediaminetetraacetic acid disodium salt dihydrate ($Na_2EDTA.2H_2O$, 99% purity) and trypsin ethylenediaminetetra acetic acid (trypsin-EDTA) were obtained from S. D. Fine Chemicals, India. Huh-7 cell lines were purchased from ATCC, Manassas, VA, USA. Water was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals and reagents of analytical grades were purchased from Himedia Pvt. Ltd., India.

Preparation of Extracts

The flowers of SP were collected from costal area of West Bengal in the month of August-September, authenticated by Botanical Survey of India, Howrah, India with a voucher specimen No. 142/BSI/2013 and were deposited for future reference. At first, the dried flowers were crushed by using a mixer grinder (Bajaj Appliances Pvt. Ltd., India) and powered accordingly. About 150 g of powder was taken in a Soxhlet apparatus and the extraction was carried out using PE, EA and ET as an extracting solvents separately for consecutive three days. Finally, the solvent was evaporated through rotary vacuum evaporator and yields of extracts in different extracting solvent were calculated. The yields were 10, 12 and 14.5% for PE, EA and ET extracts, respectively.