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Anticancer Activity of *Andrographis paniculata* and *Silybum marianum* on Five Human Cancer Cell Lines

[1]S. Singh, [1]A. Mehta, [2]S. Baweja, [1]L. Ahirwal and [1]P. Mehta
[1]Department of Botany, Dr. H. S. Gour University, Sagar (M.P.), India
[2]Department of Anatomy, Gandhi Medical College, Bhopal (M.P.), India

Corresponding Author: S. Singh, Department of Botany, Dr. H.S. Gour University, Sagar (M.P.), India

ABSTRACT

Andrographis paniculata and *Silybum marianum* are well known medicinal plants. However, to prove their efficacy for clinical utilization more scientific data are needed. Therefore, in the present study, an attempt was made to investigate the anticancer potential of hydroalcoholic extracts of *A. paniculata* and *S. marianum* and their combination (1:1). The sulphorhodamine B (SRB) assay was used to assess growth inhibition of human tumor. Five human cancer cell lines i.e., human breast adenocarcinoma (MCF-7), human cervix (SiHa), Colon (HT-29), Ovary cancer cell line (ovcar-5) and Liver (HepG2) were used for the above study. The results obtained suggest that *S. marianum* hydroalcoholic extract showed the best cytotoxic activity against all given cell lines with percentage inhibition of 21.34, 32.30, 46.56, 59.58, 36.20 for MCF 7, SiHa, HT-29, Ovcar-5 and HepG2, respectively. While, *A. paniculata* hydroalcoholic extract was found most effective against Ovcar-5 with 51.12% inhibition. The combination of both the plants (1:1) showed an intermediate result for all the cell line but, it was found to be most effective against HepG2 with 42.76% inhibition. The results obtained in the study indicate that *A. paniculata* and *S. marianum* possess significant anticancer activity and have the therapeutic potential to prevent the cancerous diseases.

Key words: *Andrographis paniculata*, *Silybum marianum,* hydroalcoholic, cancer cell lines, cytotoxic

INTRODUCTION

Cancer is the uncontrolled growth and division of cells and it leads to a mass of cells known as tumor. In the body normal cells complete their life cycle by following an orderly path of growth, division and death. The process of programmed cell death is called apoptosis. When apoptosis breaks down, it leads to cancer. Chemotherapy is a major clinical treatment used for the control of advanced stages of malignancies and metastasis. But many cancer patients seek alternative and complementary methods of treatment because of the serious side effects of chemotherapy and radiation therapy. Several herbs and plants with diversified pharmacological properties have been shown to have potential to prevent human cancers as they possess antitumor substances which have the potential to cure cancer without causing toxicity.

Andrographis paniculata is an annual herb belongs to family Acanthaceae. The plant extracts showed potent hepatoprotective (Trivedi and Rawal, 2001), vermicidal (Singh *et al*., 2009),

analgesic (Caceres *et al.*, 1997), anti-inflammatory (Wang and Zhao, 1993) and immune enhancer activity (Puri *et al.*, 1993). *Silybum marianum* is an annual or biennial erect herb belongs to the family Compositae. Different parts of this plant showed potent hepatoprotective (Fraschini *et al.*, 2002), vermicidal, anticancer (De La Puerta *et al.*, 1996), anti-inflammatory (Katiyar *et al.*, 1997) and immune enhancer activity (Lang *et al.*, 1990).

The present study was undertaken to evaluate the anticancer activity of two medicinal plants *Andrographis paniculata* and *Silybum marianum* (aerial part) and their combinational effects against five human cancer cell lines i.e. human breast adenocarcinoma (MCF-7), human cervix (SiHa), Colon (HT-29), Ovary cancer cell line (ovcar-5) and Liver (HepG2).

MATERIALS AND METHODS

Plant material: The authenticated plants materials were collected from Natural Remedies Pvt. Ltd., Bangalore (sample invoice no. D119) and confirmed at Botany Department, Dr. H.S. Gour Central University, Sagar (M.P).

Chemicals and drugs: The following drugs and chemicals were used: RPMI media, trypsin-EDTA (Sigma Chemical Co.), FBS, Penicillin, Streptomycin, PBS, Sulphorhodamine B, Di-methyl sulphoxide (DMSO). All chemicals used were of analytical grade.

Extract preparation: Andrographis paniculata and *Silybum marianum* dried and powdered aerial part were extracted separately with 70% ethanol using soxhlet apparatus. The filtrate was evaporated to dryness using a rotary evaporator (yield was 16.83% w/w and 15.04% w/w) for *A. paniculata* and *S. marianum*, respectively. The extracts were then stored below ambient temperature for further studies.

Phytochemical test: Phytochemical analysis was performed to detect various compounds such as tannins, flavonoids, alkaloids, steroids etc.

Cell lines: Five human cancer cell lines (obtained from IIIM, Jammu) were used for the study: the human breast adenocarcinoma (MCF-7), the human cervix (SiHa), Colon (HT-29), Liver (HepG2) and Ovary cancer cell line (ovcar-5).

Culturing of cell line: The cell lines were cultured in RPMI media containing 20% Foetal Bovine Serum (FBS), 2 mM l-glutamine, 100 U/ml penicillin and 100 g mL^{-1} streptomycin. All cell lines were maintained at 37°C in 5% CO_2 atmosphere with 95% humidity. Maintained cultures were passage weekly and the culture medium was changed twice a week.

Experiment: The human tumor cytotoxicity was determined by the protocols established by NCI (Monks *et al.*,1991). The Sulphorhodamine B (SRB) assay was used in this study to assess growth inhibition. The colorimetric assay estimated cell number indirectly by staining total cellular protein with the dye SRB. Single-cell suspensions were prepared by the treatment of cells with 0.5-1 mL

of 0.1% trypsin-EDTA. The viable cells were counted using a Coulter counter and diluted with RPMI medium and final densities of 100×10^4 cells mL^{-1} were obtained. Cell suspensions (100 μL $well^{-1}$) was seeded in 96-well microtiter plate containing 1 mL of media and incubated for cell attachment. After 24 h, the cells were treated with the extracts. Extracts were initially dissolved in 100% DMSO (1 mg mL^{-1} for extracts) and further diluted in RPMI medium to produce a concentration of 100 μg mL^{-1}. For combinational study (1:1) ratio, concentrations of the extracts were prepared by dissolving 50 μg of *A. paniculata* and 50 μg of *S. marianum* in 1 mL of DMSO. The control wells were filled with 100 μL of medium and plates were incubated for 48 h.

After 48 h, adherent cell cultures were fixed by adding 50 μL of cold 50% (w/v) trichloroacetic acid (TCA) and incubating for 60 min at 4°C. The supernatant was then discarded and the plates were washed 5 times with deionised water and then dried. Each microtiter well was added with 100 μL of SRB solution (0.4% w/v in 1% acetic acid) and the culture was incubated for 30 min at room temperature for complete staining reaction. Unbound SRB was removed by washing five times with 1% acetic acid and then the plates were air-dried. Bounded stain was solubilised with Tris buffer (10 mM) and the optical densities were read on an automated spectrophotometric plate reader (Molecular Devices, USA) at a wavelength of 492 nm, Optical density (OD) of SRB in each well is directly proportional to the cell number.

Statistical analysis: The experiment was performed in triplicates and the data are given as mean SRB absorbance for the calculation of inhibition±SEM. Percentage inhibition was calculated by comparing the OD of control well with that of test sample:

$$\text{Inhibition (\%)} = \left(1\frac{\text{Optical density of the treated cells}}{\text{Optical density of the control}}\right)\times100$$

RESULTS

Phytochemical study showed the presence of flavonoids, tannins, carbohydrate, cardiac glycosides, terpenes and steroids in both the plant extracts. Apart from this, alkaloids were present only in the extract of *Andrographis paniculata* while, saponins were present only in *Silybum marianum* extract (Table 1). The preliminary examination was done by observing the confluency of the test as well as control samples which showed cell growth inhibition in the test

Table 1: Phytochemical analysis of hydroalcoholic extract of *A. paniculata* and *S. marianum* extracts

Phytochemical/test	*A. paniculata*	*S. marianum*
Alkaloids	+	-
Flavonoids	++	++
Tannins	++	++
Terpenoids	+	+
Steroids	+	+
Glycosides	+	+
Carbohydrates	+	+
Saponins	-	+

+: Present, -: Absent

Table 2: Anticancer activity of hydroalcoholic extract of *A. paniculata* and *S. marianum*

		Inhibition (%)		
Cell line	Concentration of extract (μg mL^{-1})	AP	SM	AP+SM
MCF 7	100	16.04	21.34	39.22
SiHa	100	24.08	32.30	33.46
HT	100	12.42	46.56	37.10
Ovcar	100	51.12	59.58	52.38
HepG2	100	28.22	36.20	42.76

AP: *Andrographis paniculata*, SM: *Silybum marianum*

samples (Fig. 1, 2). On the basis of measurement of OD, the anticancer study suggest that *S. marianum* extract showed the best cytotoxic activity against all given cell lines (percentage inhibition was 21.34, 32.30, 46.56, 59.58, 36.20 for MCF 7, SiHa, HT, Ovcar and HepG2, respectively), while significant one was against HT and Ovcar. *Andrographis paniculata* extract was found most effective against Ovcar cell line (51.12%). Again, the combination (1:1) of both the plants showed an intermediate result for most of the cell line but the combination was most effective against the liver cell line (HepG2) which showed better activity (42.76%), as compared with the activity of both the plant extracts which gave individually (28.22 and 36.20% for *A. paniculata* and *S. marianum*, respectively) (Table 2).

DISCUSSION

Medicinal plants maintain the health and vitality of individuals and cure various diseases, including cancer without causing toxicity. These medicinal plants possess good immunomodulatory and antioxidant properties, leading to anticancer activities. The antioxidant phytochemicals protect the cells from oxidative damage. Thus, consuming a diet rich in antioxidant plant material can provide health-protective effects. These natural products are supposed to minimize DNA damage by reacting with free radicals and in this way they can prevent cancer.

Phytochemicals, such as flavonols and flavonoids were investigated to determine chemoprevention activity against cancer (Conese and Blasi, 1995). Phenols, polyphenols, flavonoids and their derivatives are ubiquitous in plants and have been found associated with the inhibition of atherosclerosis and cancer (Cirla and Mann, 2003). Recent studies have reported antitumor effects of the flavonoids, quercetin, genistein and baicalein obtained from plant extracts (Shoeb *et al*., 2006). Similarly, alkaloids like schischkinnin and montamine have been isolated from the seeds of *Centaurea schischkinii* and *Centaurea montana* which showed anticancer property (Yang and Wang, 1993). Flavonoids isolated from *Plantago* species were able to strongly inhibit the proliferation of human cancer cell lines (Galvez *et al*., 2003). Similarly, *A. paniculata* and *S. marianum* showed anticancer activity due to the presence of flavonoids, alkaloids, polyphenols which may have inhibited the protein synthesis either by damaging the DNA or by blocking at transnational level which accounted for the mortality of the cancer cells.

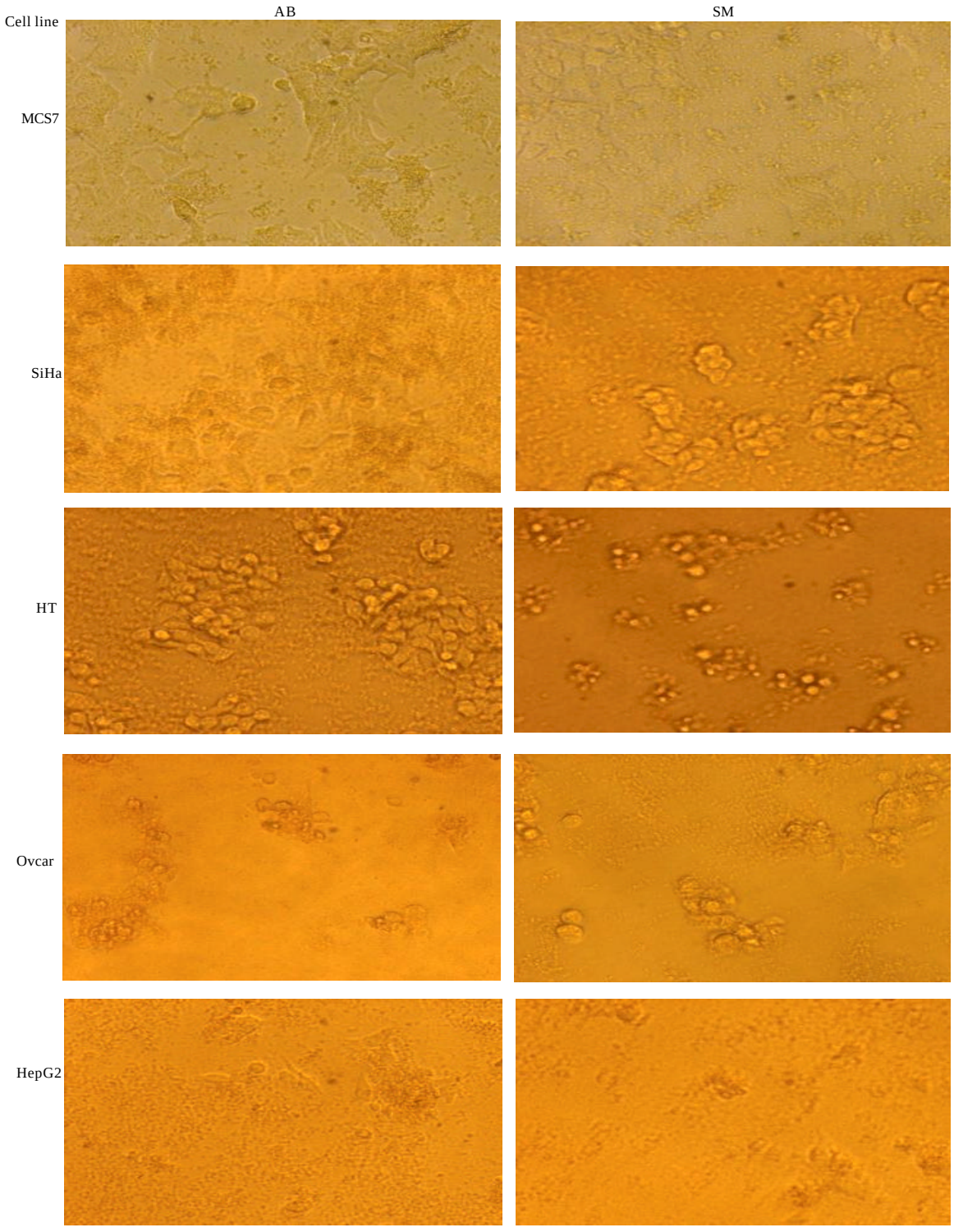

Fig. 1: Anticancer activity of hydroalcoholic extract of *A. paniculata* and *S. marianum*, AP: *A. paniculata*, SM: S. marianum

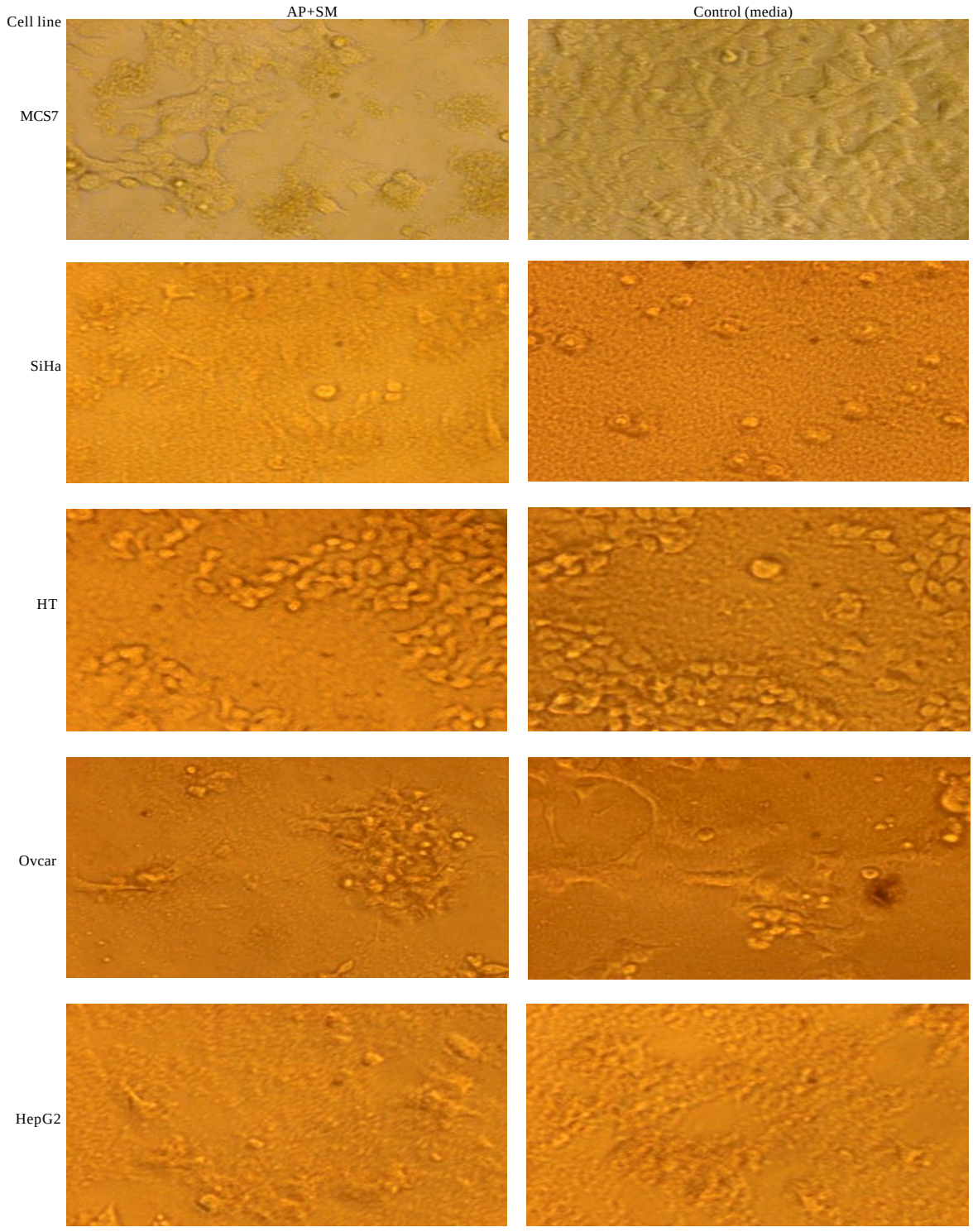

Fig. 2: Anticancer activity of combinational effects of *A. paniculata* and *S. marianum*, AP: *A. paniculata*, SM: *S. marianum*

CONCLUSION

In conclusions, the result supports the use of the plant as described in folk medicine, that the aerial plant parts can be used to treat cancer. Further studies are required to isolate the active constituents involved in the antioxidant and hepatoprotective activity of the plant.

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