Original Article

# Anticarcinogenic and antimutagenic activity of *Alstonia scholaris* on the albino mice bone marrow cells and peripheral human lymphocyte culture against methyl methane sulfonate induced genotoxicity

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## Abstract

**Background:** The use of medicinal plants in modern medicine for the prevention and treatment of cancer is an important aspect. For this reason, it is important to identify antitumor promoting agents present in medicinal plants commonly used by the human population.

**Materials and Methods:** We used *in vivo* and *in vitro* methods using chromosomal aberrations (CAs), sister chromatid exchange (SCE) and replication index (RI) as markers, exposed by methyl methanesulfonate (MMS) as well as alcoholic extract of *Alstonia scholaris* in five increasing concentrations (200, 250, 300, 350 and 400 mg/kg body weight for *in vivo* and 150, 200, 250 and 300 μg/ml of culture) and of three different durations of 24, 48 and 72 h in the presence as well absence of $S_9$ mix.

**Results:** Extracts of *Alstonia* reduces the total aberrant cells ranges from 10.0% to 41.84% and frequencies of aberration in the aberrant cells ranges from 220 to 124 against 290 aberrations causes due to MMS *in vivo*. Similarly in the *in vitro*, it reduces CAs (39.62%, 32.83%, and 38.48%) and (45.31%, 44.46%, and 38.34%) at 24, 48, and 72 h of exposure respectively; in the absence as well as presence of liver $S_9$ fraction. It also reduces SCE from 7.70 to 4.20 per cell and enhances RI from 1.45 to 1.64.

**Conclusion:** Extracts of *Alstonia* significantly reduces the number of aberrant cells and frequency of aberration per cell at each concentration and duration of exposure *in vivo*; and CAs and SCE *in vitro* and enhances RI.

**Key Words:** *Alstonia scholaris*, anticarcinogenic, antimutagenic, antitumor, ayurvedic medicine, chromosomal aberration

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## INTRODUCTION

Cancer is among the most dreaded of human diseases. It is considered as an adversary of modernization and the pattern of socioeconomic life dominated by western medicine. The use of medicinal plants in modern medicine for the prevention or treatment of cancer is an important aspect. For this reason, it is important to identify antitumor promoting agents

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present in medicinal plants commonly used by the human population, which can inhibit the initiation, progression and promotion of the tumor. Recent development of medical treatment of human disease will be intimately connected to the natural products and greater emphasis has been given towards the researches on complementary and alternative medicine that deals with cancer management.[1] Many herbs have been evaluated in clinical studies and are currently being investigated phytochemically to understand their antitumor actions against various cancers. Some herbs protect the body from cancer by enhancing detoxification functions of the body.[2] Certain biological response modifiers derived from herbs are known to inhibit the growth of cancer by modulating the activity of specific hormones and enzymes. The traditional Indian system of medicines, Ayurveda, uses about 2000 plant species, while the Chinese pharmacopeia lists over 5700 traditional medicines, most of which are of plant origin.[3]

*Alstonia scholaris* (also known as Devils tree) belonging to the family *Apocynaceae*, has been used since time immemorial in the folklore and traditional systems of medicine in India. The plant is grown in the lowland and mountain rainforests of India, the Asia-Pacific, Southern China, and Queensland. The plant is used in Ayurvedic, Unani and Siddha types of alternative medicinal systems.[4,5] The methanolic extract of this plant was found to exhibit pronounced antiplasmodial activity. The plant is reported to have antimutagenic effect.[6]

*Alstonia scholaris* is used in various Ayurvedic preparations like *Saptaparnasatvadi vati*, *Saptachadadi vati*, *Saptacchadadi kvatha* and *Saptaparna ghanasara* for uses of *A. scholaris* mainly in whooping cough, malaria, jaundice, gastric complaint, headache, asthma, stomach ache and fever. Ethanolic extract of *A. scholaris* using various concentrations under *in vitro* tests were found to have significant ($P = 0.01$) free radical scavenging and metal ion chelating properties.[6]

The bark extract of *A. scholaris* has immune-stimulating effects. The aqueous extracts at low dose induced the cellular immune response while at high dose inhibited the delayed type of hypersensitivity reaction.[7] Echitamine chloride, an indole alkaloid, extracted from the bark of *A. scholaris* has promising anticancer effect against sarcoma.[8] The plant *A. scholaris* is reported to possess *in vitro* nitric oxide scavenging activity in preliminary studies.[9] Several studies have demonstrated that plants produce potent antioxidants and represent important sources of natural antioxidants.[10,11]

The ethanolic extract of the leaves of *A. scholaris* (30, 300, 1000 and 2000 mg/kg body weight [bw]) intraperitoneally did not elicit any changes in the behavior and autonomic responses of mice compared to controls. None of the mice treated with up to 2 g/kg of the extract died during the 48 h observation period following the administration of the extract. The higher dose caused lethargy in the rats.[12] Ethnomedicinal practices suggest it to be of use in treating cancer, and preclinical studies performed with cultured neoplastic cells and tumor-bearing animals having validated these observations. In addition to the cytotoxic effects, *A. scholaris* has also been observed to possess radiomodulatory, chemomodulatory, and chemopreventive effects and free radical scavenging, antioxidant, anti-inflammatory, antimutagenic, and immunomodulatory activities, all of which are properties efficacious in the treatment and prevention of cancer.[13]

## MATERIALS AND METHODS

The whole plant of *A. scholaris* was shade dried at room temperature. Then the shade dried samples were powdered, 60 g of coarse powder was defatted with petroleum ether and extracted exhaustively with 95% of methanol at a temperature of 60°C. The extract was air dried by vacuum evaporator. Methanol extract of *A. scholaris* was dissolved in dimethyl sulfoxide (DMSO) to prepare different optimum concentrations for studies [Tables 1A and 1B].

### *In vivo* method

Albino mice 8–10 weeks old (25–35 g in weight) were exposed to different test chemicals by appropriate routes (intraperitoneal injection) and were sacrificed at sequential intervals of 16, 24, and 32 h of stipulated treatment time. Animals were treated with each test substance as shown in the tables. Three replicates of treatment were used. The central sampling interval was 24 h since cell cycle kinetics could be influenced by the test substances. The earlier and late sampling interval was adequately spaced within the range of 6–48 h. The additional dose levels were tested in subsequent experiments, the samples being taken for the scheduled duration.

#### *Preparation of slides*

Immediately after sacrifice, the bone marrows have

**Table 1A: Table of chemical concentration**

| Control | |
|---|---|
| **Positive and negative control** | **Concentrations (μg/ml)** |
| MMS | 5 |
| DMSO | 5 |

MMS: Methyl methanesulfonate, DMSO: Dimethyl sulfoxide

**Table 1B: *In vivo* concentrations of phyto-chemicals**

| Phyto-products | First dose | Second dose | Third dose | Fourth dose | Fifth dose |
|---|---|---|---|---|---|
| Alcoholic extracts of *A. scholaris in vivo* (mg/kg bw) | $AL_1$: 200 | $AL_2$: 250 | $AL_3$: 300 | $AL_4$: 350 | $AL_5$: 400 |
| Alcoholic extracts of *A. scholaris in vitro* (μg/ml) | $AL_1$: 150 | $AL_2$: 200 | $AL_3$:250 | $AL_4$: 300 | - |

AL: *A. scholaris: Alstonia scholaris*, bw: Body weight

been obtained by standard procedure and exposed to hypotonic solution and cells were fixed in Carnoy's fixative. Chromosome preparations were made from bone marrow cells following the standard procedure as described below. The stained slides were examined and metaphase cells were scored for chromosomal aberrations (CAs). Prior to sacrifice, mice were further treated with colchicines, a spindle inhibitor to arrest the cells in C-metaphase. The slides were stained with 10% aqueous Giemsa solution and about 100 bone marrow metaphase cells from each animal were scored under the code. The types of CAs considered were chromatid and chromosome gaps, breaks, and fragments, exchanges and pulverization (severely damaged cells). The reduction factors due to test chemicals treatment were calculated using the formula:

$$\text{Percentage of reduction} = \frac{\text{(Aberrant cells incontrol - aberratcells in MMS and treated cells)}}{\text{(Aberrant cells incontrol - aberrantcells in negative control)}}$$

*Control groups*

Concurrent positive and negative controls were included in this study.

Positive control

A single dose of methyl methanesulfonate (MMS), a compound known to produce CAs *in vivo,* was used as positive control showing a significant response.

## *In vitro* lymphocytes culture method

Most of the cytogenetic studies being carried out involve the examination of metaphase chromosomes. The evaluation of chromosomal damage at metaphase stage gives more precise and detailed picture of the clastogenic agent than those at anaphase or telophase stage. Human peripheral blood lymphocytes are extremely sensitive indicators of the *in vitro* assay system. The chromosomal changes (numerical and structural) were utilized for investigation of the genotoxic as well as the antigenotoxic potentiality of test chemicals. The parameters studied included CAs, sister chromatid exchanges (SCEs) and cell growth kinetics replication index (RI) both in the presence as well as in the absence of exogenous metabolic activation system.

*Preparation of $S_9$ liver/microsome fraction*

For preparing $S_9$ fraction, the standard procedures as recommended by Maron and Ames[14] were followed. Swiss albino healthy rats (Wister strain, obtained from Animal house, Biotech, Varanasi, India), each weighing about 200 g were given 0.1% (1 mg/ml) of phenobarbital in drinking water for 1-week for the induction of liver enzymatic activities. The removed livers were immediately placed in 0.15 M KCl chilled solution in the culture tube. The whole procedure was carried out at 0–4°C using sterilized solutions and glassware. The livers were washed in chilled KCl several times so as to remove the traces of hemoglobin, which inhibits enzyme activity. The washed livers were transferred to a beaker containing three volumes of 0.15 M KCl (3 ml/g wet liver) and after mincing with sterile scissors, these were homogenized by a tissue homogenizer at 4°C. The homogenate was centrifuged in a refrigeration centrifuge for 10 min at 9000 rpm. The supernatant ($S_9$ fraction) was decanted and saved as 1 ml aliquots in polypropylene storage vials and stored in liquid nitrogen till further use.

The $S_9$ mix from $S_9$ fraction was prepared fresh every time for use in the culture. The $S_9$ fraction was complemented with 8 μm of NADP, 100 μm of $Na_2HPO_4$ buffer with 7.4 pH, 0.8 ml of the $S_9$ mix was added every time along with the test chemicals in the cultures.

## Chromosomal aberrations

*Preparation of culture media*

Tissue culture medium RPMI–1640 (flow Laboratories) with L-glutamine and Hepes buffer without $NaHCO_3$ was prepared in advance and stored at 4°C, but the storage period never lasted longer than a week. About 1.574 g of the medium was dissolved in 100 ml of double distilled water by gentle shaking. Antibiotics, penicillin (100 IU/ml) and streptomycin (100 IU/ml) (Hoechst) were also added and pH was adjusted from 6.8 to 7.2 with N/10 $NaHCO_3$ and HCl. The medium was filtered and sterilized using Millipore filtration assembly by 0.45 μm Millipore filters. The filtered medium was then stored in sterilized and tightly capped glass bottles.

*Collection of blood samples*

Peripheral blood from the healthy donors was taken fresh every time through veinal puncture under

aseptic conditions (disposable needle and disposable syringes, Unitech) and Heparin (500 IU/ml; Micro Lab) was used as anticoagulant. The tightly capped glass vials were gently mixed and stored at 4°C for half an hour to separate blood cells from plasma.

*Setting of the cultures*

Lymphocyte culture was carried out by adding 0.8 ml of plasma containing white blood cells in 4.5 ml of culture medium supplemented with 0.1 ml phytohemagglutinin–P (PHA–P, Micro lab) and 15% fetal calf serum (Gibco). The culture vials were then tightly capped to avoid loss $CO_2$ of and after gently mixing, culture tubes were incubated at 37°C in dark and colchicine was added 2 h prior to harvesting for arresting the cells at metaphase stage.

*Harvesting of the cultures*

After appropriate durations, the cultures were taken out from the incubator and their contents, after gentle shaking, were transferred to a centrifuge tube, the cells were spun down by centrifugation for 10 min, at 1200 rpm. Pellets were saved by discarding the supernatant. Hypotonic treatment (0.075 M KCl) was given for 10–12 min at 37°C and the cells were recollected by centrifugation. The cell pellet was suspended in 5 ml freshly prepared chilled fixative (3:1; methanol: acetic acid), which was added drop by drop with a Pasteur pipette with continuous shaking to avoid formation of clots. In order to ensure the proper fixation, the cells were kept suspended in the fixative for a minimum period of 1 h but preferably overnight. Two or three changes with fresh fixative were given before preparing the slides.

*Slide preparation and staining*

After giving final washing in the fixative, the cells were re-suspended in 0.2 ml of fresh fixative. Two or three drops of cell preparation were dropped on clean, grease free, prechilled and wet microscopic slides and air dried. One-day-old slides were stained with Giemsa (Sigma) for 15 min and rinsed in 95% alcohol and finally in absolute alcohol for proper differentiation, after air-drying these slides were dipped in xylene for 5 min before mounting in dibutyl phthalate xylene (DPX).

*Analysis of the cells*

In order to avoid the bias in scoring of the chromosomal anomalies before and after treatment of different test chemicals all slides were coded prior to scoring. A total of 300 well-spread metaphase were analyze for each concentration of the test chemicals and for each time duration to analyzed various chromosome and chromatid type aberrations by using the method as described by Evans.[15]

## Sister chromatid exchange analysis

Sister chromatid exchange is a sensitive rapid and objective method of observing reciprocal exchange between sisters chromatid. This method depends upon the phenomenon of 5-bromo-2-deoxyuridine (BrdU) incorporation into DNA in place of thymidine. After two rounds of cell division, the chromatids were labeled with BrdU and consequently differentially stained with Hoechst stain. The BrdU incorporation quenches the fluorescence of 33258 Hoechst. Therefore, the light energy is absorbed but not emitted by such dyes, which results in the reduced staining of chromatid with Giemsa.[14]

*Labeling of chromosomes with 5-bromo-2-deoxyuridine*

Sister chromatid exchange analysis was carried out following the standard procedure of Latt and Wohlleb.[16] The cells in the culture were exposed to nucleoside, BrdU (Sigma) after 24 h of culture initiation at the final concentration of 2 μg/ml. The culture vials were tightly capped and covered with aluminum foil to avoid light exposure and incubated at 37°C for another 48 h in the dark.

*Slide preparation and staining*

After 2 h of colchicines treatment, the cultures were harvested and processed following the same procedure as desired for the CA analysis. For the differential staining of SCEs the methods of Latt *et al*.[17] with slight modifications were followed. 1-day-old slides were dipped in 0.5 μg/ml of 33258 Hoechst stain (Sigma) dissolved in double distilled water in horizontal coupling jar. The slides were then put in a flat glass dish with the layer of cells facing upwards. These were covered by thick layer (2–3 cm) of phosphate buffer (pH 6.8) and exposed to ultraviolet lamp (15W, 254 μm, Philips) from a distance of 10 to 15 cm for 30–45 min. The slides were taken out from the buffer, washed twice in double distilled water and air dried. These were then incubated in 2X SSC (0.3 M NaCl, 0.03M sodium citrate, pH 7.0) at 65°C in water bath for 90 min using vertical couplin jars. The slides were taken out and rinsed in distilled water. The air-dried slides were then stained with Giemsa for 20 min and rinsed in 90% alcohol, followed by rinsing in absolute alcohol. The dried slides were dipped in xylene for 5 min and mounted in DPX.

*Analysis of the cells*

All slides were coded prior to scoring so as to avoid any ambiguity. Around 50 metaphases (25 metaphases/donor) with differentially stained chromatid were scored for each test chemical treatment in the absence of $S_9$ mix and 50 metaphases were scored for each treatment in the presence of $S_9$ mix. The

interstitial exchanges between two sister chromatid were scored as two exchange, and the terminal exchanges were scored as a single exchange. Student's *t*-test was applied for calculating the significance of difference between the treated and the controls.

### Cell cycle kinetics analysis

The cells undergoing first ($M_1$) second ($M_2$) and third ($M_3$) divisions were detected by studying the BrdU labeled differentially stained chromosomes, following the method of Crossen and Morgan.[18] The cells with both the chromatids being darkly stained were scored as $M_{1\ cells}$, those with one dark and one lightly stained chromatid as $M_{2\ cells}$ and those having mixture of both the differentially stained and uniformly stained chromatids were scored as $M_3$ metaphase. Around 100 well-spread metaphase were scored for each concentration and each treatment durations from each donor in the absence as well as in the presence of $S_9$ mix. The RI was calculated according to the formula of Tice *et al*.[19] as given below. The deviation from the controls was determined by using Chi-square ($\chi^2$) test.

$$\text{R.I} = \frac{(M_1X1)+(M_2X2)+(M_3X3)}{100}$$

### Statistical analysis

2 × 3 Chi-square test ($\chi^2$) for homogeneity test of variance was used to analyses the cell growth kinetics exchange with the normal control. The level of significance was tested from standard statistical tables of Fisher and Yates.[20]

Student two-tailed *t*-test was used for calculating the statistical significance in SCE and CA by comparing the effect induced by different test chemicals with the respective control.

The statistical significance was calculated from Fisher and Yates table at $(n_1+n_2-2)$ degree of freedom at 0.05% level of significance.

## RESULTS

### *In vivo* effects

In this study, the albino mice were exposed till 16 h of treatments and found that the percentage of aberrant cells were 9.9, 8.7, 8.2, 7.3 and 6.4, respectively at five different concentrations of *Alstonia* extract respectively against 11.0% of aberrant cells induced by MMS in positive control. Fragments types of aberrations were most prominent, followed by breaks and gaps, whereas exchanges were almost negligible. In terms of percentage reduction in the frequencies of aberrant cells, the observed values are 10.0, 20.90, 25.45, 33.63 and 41.87 against five different concentrations of *Alstonia* extract respectively. The maximum effect of *Alstonia* extract was 41.87% at the fifth concentration of the extract [Table 1C and Figure 1].

The effect on the total number of frequencies per thousand cells was 182, 147, 138, 125 and 105 at five consecutive concentrations of *Alstonia* extract against 209 when treated with MMS alone. The normal values were 25 for distilled water treatment and 28 and 28 for DMSO and *Alstonia* extract only treatment [Table 2]. When the treatment durations were increased to 24 h, the effects were still following the same trend, showing increasing values. The values are 11.5%, 11.0%, 10.3%, 9.5% and 8.7% for five concentrations of *Alstonia* extract respectively against 13.5% of MMS treatment only. The values of normal are 3.0%, 3.0%, and 2.8% respectively for pure water, DMSO and *Alstonia* extract only. The fifth concentration only shows a noticeable effect on the percentage reductions of aberrant cells [Table 3 and Figure 2].

Effect of *Alstonia* extract on the frequency of aberrations per cell and total aberrations were also not so much promising. The total aberrations per thousand

**Table 1c: Effect of with alcoholic extracts of *A. scholaris* on the frequency of cells with chromosome aberrations induced by MMS**

| Treatment | ALE (Y/kg bw) | Cell with pulverized chromosome | Types of chromatic aberrations | | | | Aberrant cell | | Reduction (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Gaps | Breaks | Fragments | Exchanges | Number | % | |
| $DH_2O$ | 0 | 00 | 02 | 03 | 18 | 00 | 21 | 2.1 | |
| $DH_2O$ + DMSO | 0 | 00 | 03 | 01 | 24 | 00 | 25 | 2.5 | |
| MMS | 0 | 17 | 52 | 35 | 53 | 05 | 110 | 11.0 | |
| ALE | $AL_5$ | 00 | 05 | 02 | 24 | 00 | 26 | 2.6 | |
| MMS + ALE | $AL_1$ | 13 | 51 | 36 | 46 | 04 | 99 | 9.9 | 10.00 |
| | $AL_2$ | 12 | 48 | 33 | 39 | 03 | 87 | 8.7 | 20.90 |
| | $AL_3$ | 10 | 43 | 32 | 38 | 02 | 82 | 8.2 | 25.45 |
| | $AL_4$ | 07 | 40 | 30 | 35 | 01 | 73 | 7.3 | 33.63 |
| | $AL_5$ | 05 | 35 | 28 | 31 | 00 | 64 | 6.4 | 41.81 |

ALE or AL: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methanesulfonate 5 μg/ml/kg bw at 16 h of treatment. Calculations were made excluding the gaps type of an aberration and at <0.05 probability. Y/kg bw is the concentration of alcoholic extracts of *A. scholaris*. DMSO: Dimethyl sulfoxide, *A. scholaris*: *Alstonia scholaris*, bw: Body weight

cells were 219, 194, 179, 152 and 141 for *Alstonia* extract along with MMS against 265 due to MMS only as positive control [Table 4]. At 32 h exposure, the percent aberrant cells observed were 15.0% for MMS alone, and 13.0%, 12.2%, 11.2%, 10.4%, 9.0% for five different concentrations of *Alstonia* extract along with MMS, whereas the values for normal control was 2.3, whereas for DMSO and *Alstonia* extract alone, the values were 2.0% and 2.6% respectively. In terms of the effects on the percent reduction in aberrant cells, the range was from 13.33%, 18.66%, 25.33%, and 30.66% to 40.00% respectively. These values show a significant effect of *Alstonia* extract on the number and percentage of aberrant cells. It also shows almost dose-dependent relationship. More chromosomal exchange types of aberrations were seen in contrast to the previous two durations of treatment [Table 5 and Figure 3].

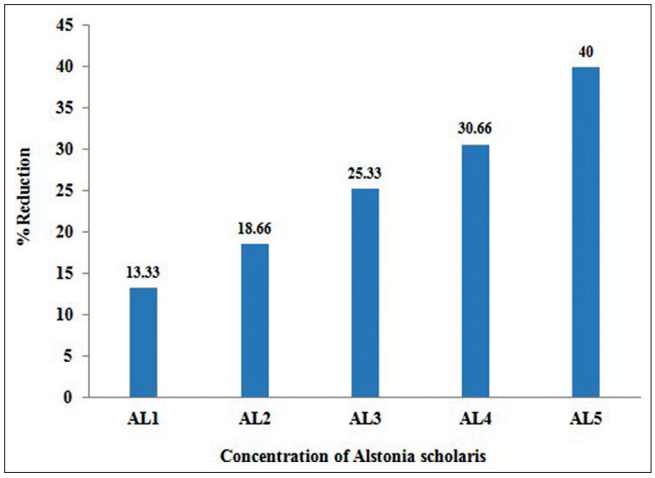


**Figure 1:** *In vivo* effect of alcoholic extracts of *Alstonia scholaris* at 16 h

**Table 2: Effect of with alcoholic extracts of *A. scholaris* on the total number and types of frequency of cells with chromosome aberrations induced by MMS**

| Treatment | ALE concentrations (Y/kg bw) | Cell with aberration | | | | | | | Total number of aberration |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6-9 | |
| $DH_2O$ | 0 | 979 | 19 | 03 | 00 | 00 | 00 | 00 | 25 |
| $DH_2O$ + DMSO | 0 | 975 | 22 | 03 | 00 | 00 | 00 | 00 | 28 |
| MMS | 0 | 890 | 68 | 21 | 05 | 06 | 04 | 05 | 209 |
| ALE | $AL_5$ | 974 | 24 | 02 | 00 | 00 | 00 | 00 | 28 |
| MMS + ALE | $AL_1$ | 901 | 66 | 17 | 03 | 05 | 04 | 04 | 182 |
| | $AL_2$ | 913 | 61 | 15 | 03 | 03 | 02 | 03 | 147 |
| | $AL_3$ | 918 | 57 | 14 | 02 | 04 | 03 | 02 | 138 |
| | $AL_4$ | 927 | 48 | 15 | 03 | 03 | 02 | 02 | 125 |
| | $AL_5$ | 936 | 42 | 13 | 04 | 03 | 01 | 01 | 105 |

ALE or AL: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methane sulfonate 5 μg/ml/kg bw at 16 h of treatment. Calculations were made excluding the gaps type of an aberration and at <0.05 probability. The animals were sacrificed 16 h after MMS treatment 1000 cells from 10 animals were analyzed for each point. Y/kg bw is the concentration of alcoholic extracts of *A. scholaris*. DMSO: Dimethyl sulfoxide, *A. scholaris: Alstonia scholaris*, bw: Body weight

Table 5 and Figure 3].

The total aberrant chromosomal frequencies per thousand cells recorded were 290 for MMS and about 220, 193, 170, 147 and 124 for *Alstonia* extract along with MMS for five different concentrations of *Alstonia* extract. These frequencies show the effects of *Alstonia* extract in significantly reducing the total aberrations as well as aberrations per cell as shown in Table 6.

### *In vitro* effects

Treatment with MMS results in clastogenic abnormalities as observed in percent metaphase aberration, types of aberrations and aberration per cell viz., 39.75%, 67.00%, 69.50% and 0.40, 0.67 and 0.70 aberration per cell, whereas the control the normal and the DMSO plus *Alstonia* extract values are 04.00, 04.50 per cell at single standard dosage and for three various durations are 24, 48 and 72 h. *Alstonia* extract bring down aberrations from 39.75% to 32.50%, 28.75%, 26.25% and 24.00% with four consecutive dosages of *Alstonia* extract at 24 h of duration, whereas at 48 h, it is lowered from 67.00% to 50.50%, 43.35%, 49.00% and 45.00% respectively by first to fourth concentrations of *Alstonia* extract. Similar trend was noticed when the treatment durations were increased to 72 h. These values show linear increasing trend with

**Table 3: Effect of with alcoholic extracts of *A. scholaris* on the frequency of cells with chromosome aberrations induced by MMS**

| Treatment | ALE concentration (Y/kg bw) | Cell with pulverized chromosome | Types of chromatic aberrations | | | | Aberrant cell | | Percentage of reduction |
|---|---|---|---|---|---|---|---|---|---|
| | | | Gaps | Breaks | Fragments | Exchange | Number | % | |
| $DH_2O$ | 00 | 00 | 01 | 01 | 29 | 00 | 30 | 3.0 | |
| $DH_2O$ + DMSO | 00 | 00 | 02 | 03 | 27 | 00 | 30 | 3.0 | |
| MMS | 00 | 20 | 56 | 55 | 53 | 07 | 135 | 13.5 | |
| ALE | $AL_5$ | 00 | 03 | 04 | 24 | 00 | 28 | 2.8 | |
| MMS + ALE | $AL_1$ | 14 | 49 | 49 | 47 | 05 | 115 | 11.5 | 14.81 |
| | $AL_2$ | 11 | 52 | 50 | 45 | 04 | 110 | 11.0 | 18.51 |
| | $AL_3$ | 09 | 47 | 47 | 44 | 03 | 103 | 10.3 | 23.70 |
| | $AL_4$ | 07 | 41 | 44 | 42 | 02 | 95 | 9.5 | 29.62 |
| | $AL_5$ | 04 | 36 | 40 | 41 | 02 | 87 | 8.7 | 35.55 |

ALE or AL: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methane sulfonate 5 μg/ml/kg bw at 24 h of treatment. Calculations were made excluding the gaps type of a aberration and at <0.05 probability. Y/kg bw is the concentration of alcoholic extracts of *A. scholaris*. DMSO: Dimethyl sulfoxide, *A. scholaris: Alstonia scholaris*, bw: Body weight

dosages, but it does not depend on dose-durations. The maximum percentage reductions in the aberrations were 39.62 for 24 h, which were 32.83 and 38.48 for 48 and 72 h respectively [Table 7].

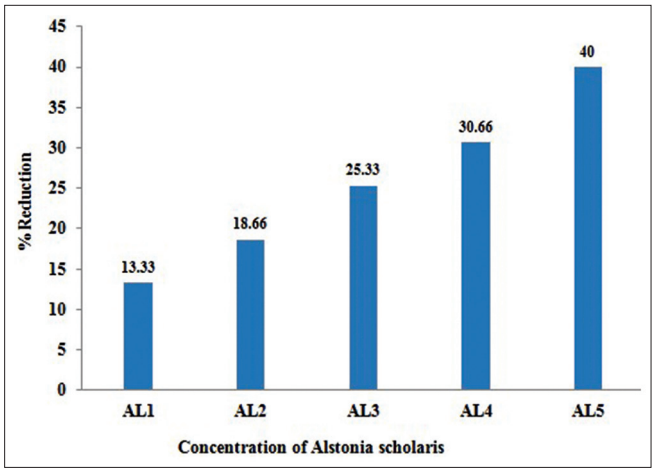


**Figure 2:** *In vivo* effect of alcoholic extracts of *Alstonia scholaris* at 24 h

**Table 4: Effect of with alcoholic extracts of *A. scholaris* on the total number and types of frequency of cells with chromosome aberrations induced by MMS**

| Treatment | ALE concentration (Y/kg bw) | Cell with aberration | | | | | | | Total number of aberration |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6-9 | |
| $DH_2O$ | 00 | 970 | 28 | 02 | 00 | 00 | 00 | 00 | 32 |
| $DH_2O$ + DMSO | 00 | 970 | 26 | 04 | 00 | 00 | 00 | 00 | 34 |
| MMS | 00 | 865 | 84 | 23 | 07 | 08 | 07 | 06 | 265 |
| ALE | $AL_5$ | 972 | 26 | 02 | 00 | 00 | 00 | 00 | 30 |
| MMS + ALE | $AL_1$ | 885 | 73 | 21 | 05 | 06 | 05 | 05 | 219 |
| | $AL_2$ | 890 | 74 | 19 | 04 | 05 | 05 | 03 | 194 |
| | $AL_3$ | 897 | 71 | 17 | 05 | 03 | 03 | 04 | 179 |
| | $AL_4$ | 905 | 68 | 14 | 05 | 04 | 02 | 02 | 152 |
| | $AL_5$ | 913 | 63 | 12 | 04 | 03 | 03 | 02 | 141 |

ALE or AL: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methanesulfonate 5 μg/ml/kg bw at 24 h of treatment. Calculations were made excluding the gaps type of an aberration and at <0.05 probability. The animals were sacrificed 24 h after MMS treatment 1000 cells from 10 animals were analyzed for each point. Y/kg bw is the concentration of alcoholic extracts of *A. scholaris*. DMSO: Dimethyl sulfoxide, *A. scholaris: Alstonia scholaris,* bw: Body weight

When culture was setup along with metabolic activation system (+$S_9$ mix), the effect of MMS increased. Similarly, the effects of *Alstonia* extract also lowered the clastogenic activity of MMS. These values show linearly increasing trend with doses [Table 8]. The maximum effective percentage reductions were 45.31%, 44.46%, and 38.34% for 24, 48 and 72 h respectively. The highest reduction on clastogeny of cells was noticed at 24 h durations; though the other values were also statistically significant.

The experiment were conducted for SCEs assay [Tables 9 and 10], the reduction was evident both in the absence as well as in the presence of metabolic activation; there being a lowering of the mean range and the total SCEs and SCE per cell from 07.70 to 04.30 and from 7.20 to 04.20. For conducting SCE assay, only 48 h of cultures were done, and 50 metaphases were scored for counting the number of exchanges.

The effects of *Alstonia* extract on RI [Tables 11 and 12] show an elevated level when compared with the MMS treatment that is, rising from 1.45 to 1.58, though still lower than the normal level of 1.69. The effect, after treatment with metabolic activation system shows to elevated from 1.45 to 1.64 that is, being much effective than one without metabolic activation system. Therefore, we observed that *Alstonia* extract has potent anti-clastogenic activities in these experiments.

## DISCUSSION

Cancer cells are "immortal" that is, they have lost their growth restraining mechanisms and so multiply out of control. This results from alteration of cellular DNA or genetic material, which can be an inherited defect. It was found that free radical damage is the cause of these genetic mutations. When DNA or genetic material is involved in free radical reactions, mutations or genetic alteration can result. Free radical chain reactions are stopped

**Table 5: Effect of with alcoholic extracts of *A. scholaris* on the frequency of cells with chromosome aberrations induced by MMS**

| Treatment | ALE concentration (Y/kg bw) | Cell with pulverized chromosome | Types of chromatic aberrations | | | | Aberrant cell | | Percentage of reduction |
|---|---|---|---|---|---|---|---|---|---|
| | | | Gaps | Breaks | Fragments | Exchange | Number | % | |
| $DH_2O$ | 00 | 0 | 01 | 01 | 22 | 00 | 23 | 2.3 | |
| $DH_2O$ + DMSOO | 00 | 0 | 02 | 02 | 18 | 00 | 20 | 2.0 | |
| MMS | 00 | 25 | 42 | 52 | 62 | 11 | 150 | 15.0 | |
| ALE | $AL_5$ | 0 | 05 | 02 | 24 | 00 | 26 | 2.6 | |
| MMS + ALE | $AL_1$ | 21 | 40 | 49 | 54 | 06 | 130 | 13.0 | 13.33 |
| | $AL_2$ | 15 | 38 | 52 | 51 | 04 | 122 | 12.2 | 18.66 |
| | $AL_3$ | 11 | 36 | 50 | 49 | 02 | 112 | 11.2 | 25.33 |
| | $AL_4$ | 09 | 34 | 46 | 47 | 02 | 104 | 10.4 | 30.66 |
| | $AL_5$ | 07 | 35 | 40 | 40 | 03 | 90 | 9.0 | 40.00 |

ALE or AL: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methane sulfonate 5 μg/ml/kg bw at 32 h of treatment. Calculations were made excluding the gaps type of a aberration and at <0.05 probability. Y/kg bw is the concentration of alcoholic extracts of *A. scholaris*. DMSO: Dimethyl sulfoxide, *A. scholaris: Alstonia scholaris*, bw: Body weight

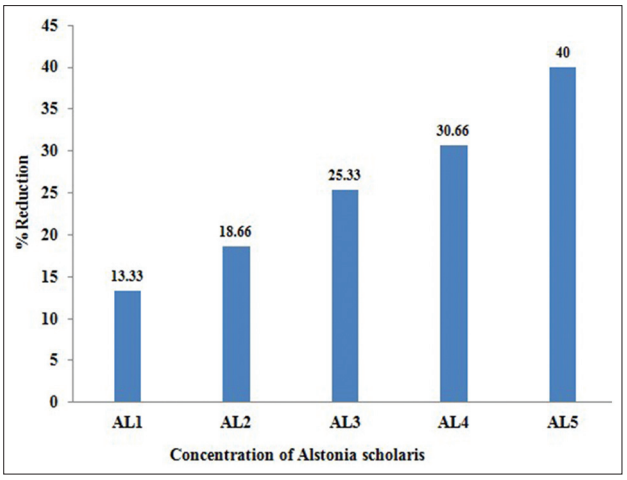


**Figure 3:** *In vivo* effect of alcoholic extracts of *Alstonia scholaris* at 32 h

by the action of antioxidants. In our experiment, the protective effects of alcoholic extracts of *A. scholaris* may be due to this reason that is, quenching the free radicals that were generated due to mutagen and carcinogen.

The anticancer effect of various doses of an alkaloid fraction of *A. scholaris* (ASERS), was studied *in vitro* in cultured human neoplastic cell lines (HeLa, HepG2, HL60, KB and MCF-7) and in Ehrlich ascites carcinoma-bearing mice.[21] Treatment of HeLa cells with 25 g/ml ASERS resulted in a time-dependent increase in the antineoplastic activity and the greatest activity was observed when the cells were exposed to ASERS for 24 h.[21] However, exposure of cells to ASERS for 4 h resulted in 25% viable cells and hence this time interval was considered to be the optimum time for treatment and further studies were carried out using this time.[21] Treatment of various cells with ASERS resulted in a concentration-dependent decline in the viable cells and a nadir was reached at 200 g/ml in the entire cell lines studied, we too have noticed similar trend in our *in vitro* experiment. The inhibitory concentration 50% was found to be 5.53, 25, 11.16, 10 and 29.76 g/ml for HeLa, HePG2, HL60, KB and MCF-7 cells, respectively. Similarly, administration of ASERS, once daily for nine consecutive days to the tumor-bearing mice caused a dose-dependent remission of the tumor up to 240 mg/kg bw, where the greatest antitumor effect was observed. Since 240 mg/kg ASERS showed toxic manifestations, the next lower dose of 210 mg/kg was considered as the best effective dose, in which 20% of the animals survived up to 120 days posttumor cell inoculation as against no survivors in the saline-treated control group.[21]

The chemomodulatory activity of *A. scholaris* extract was studied in combination with berberine

**Table 6: Effect of with alcoholic extractsof *A. scholaris* on the total number and types of frequency of cells with chromosome aberrations induced by MMS**

| Treatment | ALE concentration (Y/kg bw) | Cell with aberration | | | | | | | Total number of aberration |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6-9 | |
| $DH_2O$ | 00 | 977 | 21 | 02 | 00 | 00 | 00 | 00 | 25 |
| $DH_2O$ + DMSO | 00 | 980 | 18 | 02 | 00 | 00 | 00 | 00 | 22 |
| MMS | 00 | 850 | 94 | 24 | 11 | 08 | 07 | 06 | 290 |
| ALE | $AL_5$ | 974 | 24 | 02 | 00 | 00 | 00 | 00 | 28 |
| MMS+ALE | $AL_1$ | 870 | 90 | 20 | 07 | 05 | 05 | 03 | 220 |
| | $AL_2$ | 878 | 88 | 19 | 05 | 03 | 05 | 02 | 193 |
| | $AL_3$ | 888 | 85 | 16 | 03 | 03 | 03 | 02 | 170 |
| | $AL_4$ | 896 | 82 | 14 | 02 | 02 | 03 | 01 | 147 |
| | $AL_5$ | 910 | 71 | 11 | 03 | 03 | 02 | 00 | 124 |

ALE or AL: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methane sulfonate 5 μg/ml/kg bw at 32 h of treatment. Calculations were made excluding the gaps type of an aberration and at <0.05 probability. The animals were sacrificed 32 h after MMS treatment 1000 cells from 10 animals were analyzed for each point. Y/kg bw is the concentration of alcoholic extracts of *A. scholaris*. DMSO: Dimethyl sulfoxide, *A. scholaris: Alstonia scholaris*, bw: Body weight

hydrochloride (BCL), a topoisomerase inhibitor, in Ehrlich ascites carcinoma-bearing mice. The best effect was observed when 180 mg/kg of *A.scholaris* extract (ASE) was combined with 6 or 8 mg/kg of BCL, where an increase in the antineoplastic activity was reported.[9]

The possible chemopreventive and anti-oxidative properties of this medicinal plant on two-stage process of skin carcinogenesis induced by a single application of 7,12-dimethyabenz (a) anthracene (100 lg/100 ll acetone), whereas 2 weeks later, these are promoted by repeated application of croton oil (1% increase in incidence, tumor yield, tumor burden and cumulative number of papillomas). These changes are found to be higher in the carcinogen treated control (without ASE treatment) as compared to experimental animals (ASE treated). Furthermore, a significant increase in reduced glutathione, superoxide dismutase, and catalase level but decrease in lipid peroxidation was measured in ASE administered experimental groups than the carcinogen treated controls.[22]

Chemopreventive agents can be targeted by intervention at the initiation, promotion, or progression stage of multistage carcinogenesis.[23,24] The intervention of cancer at the promotion stage, however, seems to be the most appropriate and practical. The major reason for that is, relates to the fact that tumor promotion is a reversible event at least in early stages and requires repeated and prolonged exposure of a promoting agent.[25]

The hydroalcoholic extract of *A. scholaris* protected against benzo(a) pyrene-induced forestomach

**Table 7: Analysis of CAs after treatment with MMS along with alcoholic extracts of *A. scholaris in vitro* in the absence of $-S_9$ mix**

| Treatments | Durations (h) | Metaphase scored | Percentage aberration metaphase | | Types of aberration (%) | | | Aberration/ cell±SE |
|---|---|---|---|---|---|---|---|---|
| | | | Including gap | Excluding gap | Chromatid | Chromosome | Total | |
| MMS | 24 | 200 | 25.00 | 23.50 | 27.50 | 12.25 | 39.75 | 0.40±0.04 |
| | 48 | 200 | 40.25 | 36.25 | 43.75 | 23.25 | 67.00 | 0.67±0.06 |
| | 72 | 200 | 42.75 | 37.35 | 48.25 | 21.25 | 69.50 | 0.70±0.06 |
| MMS + $AL_1$ | 24 | 200 | 20.70 | 15.00 | 21.00 | 11.50 | 32.50 | 0.33±0.03 |
| | 48 | 200 | 30.25 | 27.50 | 33.25 | 17.25 | 50.50 | 0.51±0.05 |
| | 72 | 200 | 33.50 | 29.70 | 36.50 | 19.50 | 56.00 | 0.56±0.05 |
| MMS + $AL_2$ | 24 | 200 | 16.50 | 14.25 | 18.50 | 10.25 | 28.75 | 0.29±0.03 |
| | 48 | 200 | 26.75 | 24.25 | 28.00 | 15.35 | 43.35 | 0.43±0.04 |
| | 72 | 200 | 30.00 | 27.50 | 33.25 | 18.25 | 51.50 | 0.52±0.05 |
| MMS + $AL_3$ | 24 | 200 | 15.75 | 14.00 | 16.50 | 9.75 | 26.25 | 0.26±0.03 |
| | 48 | 200 | 24.20 | 22.30 | 24.25 | 14.75 | 49.00 | 0.49±0.04 |
| | 72 | 200 | 28.00 | 24.50 | 31.50 | 16.25 | 47.75 | 0.48±0.04 |
| MMS + $AL_4$ | 24 | 200 | 14.50 | 13.50 | 14.50 | 9.50 | 24.00 | 0.24±0.03 |
| | 48 | 200 | 23.00 | 21.50 | 22.25 | 12.75 | 45.00 | 0.45±0.04 |
| | 72 | 200 | 26.50 | 22.75 | 28.50 | 14.25 | 42.75 | 0.43±0.04 |
| Control | | | | | | | | |
| Normal | 72 | 200 | 3.50 | 1.50 | 2.50 | 1.50 | 4.00 | 0.04±0.01 |
| DMSO + $AL_2$ | 72 | 200 | 4.50 | 1.70 | 3.00 | 1.50 | 4.50 | 0.05±0.01 |

A AL1-4:Concentrations of alcoholic extract of *A.scholaris* SE: Standard error, DMSO: Dimethyl sulfoxide, *A. scholaris: Alstonia scholaris*, CAs: Chromosomal aberrations. AL or ALE: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methane sulfonate 5 μg/ml culture. Calculations were made excluding the gaps type of an aberration and at <0.05 probability

**Table 8: Analysis of CAs after treatment with MMS along with alcoholic extracts of *A. scholaris in vitro* in the presence of $+S_9$ mix**

| Treatments | Durations (h) | Metaphase scored | Percentage aberration metaphase | | Types of aberration (%) | | | Aberration/ cell±SE |
|---|---|---|---|---|---|---|---|---|
| | | | Including gap | Excluding gap | Chromatid | Chromosome | Total | |
| MMS | 24 | 200 | 22.00 | 19.00 | 23.50 | 8.50 | 32.00 | 0.32±0.04 |
| | 48 | 200 | 37.50 | 32.25 | 40.00 | 20.50 | 60.50 | 0.61±0.06 |
| | 72 | 200 | 39.25 | 33.00 | 45.25 | 21.25 | 66.50 | 0.67±0.06 |
| MMS + $AL_1$ | 24 | 200 | 15.50 | 12.75 | 18.25 | 7.25 | 25.50 | 0.26±0.03 |
| | 48 | 200 | 27.75 | 25.25 | 31.50 | 15.00 | 46.50 | 0.47±0.04 |
| | 72 | 200 | 30.25 | 27.50 | 35.00 | 17.50 | 52.50 | 0.53±0.05 |
| MMS + $AL_2$ | 24 | 200 | 14.25 | 12.50 | 16.50 | 7.00 | 23.50 | 0.24±0.03 |
| | 48 | 200 | 23.50 | 22.25 | 25.50 | 13.25 | 38.75 | 0.39±0.04 |
| | 72 | 200 | 26.25 | 23.50 | 31.25 | 15.50 | 46.75 | 0.47±0.04 |
| MMS + $AL_3$ | 24 | 200 | 14.50 | 13.25 | 14.25 | 6.50 | 20.75 | 0.21±0.03 |
| | 48 | 200 | 22.50 | 21.35 | 21.50 | 12.75 | 34.25 | 0.34±0.04 |
| | 72 | 200 | 25.75 | 21.50 | 30.25 | 14.75 | 45.00 | 0.45±0.04 |
| MMS + $AL_4$ | 24 | 200 | 13.75 | 12.35 | 11.50 | 6.00 | 17.50 | 0.18±0.03 |
| | 48 | 200 | 21.50 | 20.00 | 22.35 | 11.25 | 33.60 | 0.34±0.04 |
| | 72 | 200 | 24.50 | 19.75 | 27.50 | 13.50 | 41.00 | 0.41±0.04 |
| Control | | | | | | | | |
| Normal | 72 | 200 | 2.30 | 1.80 | 1.75 | 1.50 | 3.25 | 0.03±0.01 |

AL1-4: Concentrations of alcoholic extract of *A.scholaris* SE: Standard error, DMSO: Dimethyl sulfoxide, *A. scholaris: Alstonia scholaris*, CAs: Chromosomal aberrations. AL or ALE: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methane sulphonate 5 μg/ml culture. Calculations were made excluding the gaps type of an aberration and at <0.05 probability

carcinoma in the female mice when the extract is added to drinking water at doses of 1, 2 and 4 mg/ml for 2 weeks before the treatment, during the treatment and 2 weeks after the carcinogen exposure. These doses reduced tumor multiplicity by 21.43, 28.57 and 50%, respectively. The greatest protection was afforded by the highest dose, which reduced tumor incidence by 6.67%.[26] Tumor multiplicity incidence was significantly reduced (91.93% with extract vs. 100% in benzo(a) pyrene-treated mice) by 4 mg/ml dose that was added to the drinking water during the postinitiation period, starting at 48 h after the last dose of benzo(a) pyrene (posttreatment) these continued for 8 weeks.

**Table 9: Analysis of SCE after treatment with MMS along with alcoholic extracts of *A. scholaris in vitro*, in the absence of $-S_9$ mix**

| Treatment | Duration (h) | metaphase scored | Total | Range | SCE/ cell±SE |
|---|---|---|---|---|---|
| MMS | 48 | 50 | 385 | 1-11 | 7.70±1.50 |
| MMS + $AL_1$ | 48 | 50 | 330 | 1-11 | 6.60±1.50 |
| MMS + $AL_2$ | 48 | 50 | 275 | 1-10 | 5.50±1.50 |
| MMS + $AL_3$ | 48 | 50 | 245 | 1-10 | 4.90±1.50 |
| MMS + $AL_4$ | 48 | 50 | 215 | 1-10 | 4.30±1.50 |
| Control | | | | | |
| Normal | 48 | 50 | 91 | 0-4 | 1.82±1.00 |
| DMSO | 48 | 50 | 94 | 0-5 | 1.88±1.00 |
| DMSO + $AL_2$ | 48 | 50 | 90 | 0-4 | 1.80±1.00 |

SCE: Sister chromatid exchange, DMSO: Dimethyl sulfoxide, SE: Standard error. AL or ALE: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methane sulphonate 5 μg/ml culture. Calculations were made at <0.05 probability. *A. scholaris: Alstonia scholaris*

**Table 10: Analysis of SCE after treatment with MMS along with alcoholic extracts of *A. scholaris in vitro*, in the presence of $+S_9$ mix**

| Treatment | Duration (h) | Metaphase scored | Total | Range | SCE/ cell±SE |
|---|---|---|---|---|---|
| MMS | 48 | 50 | 360 | 3-12 | 7.20±1.50 |
| MMS + $AL_1$ | 48 | 50 | 310 | 1-11 | 6.20±1.50 |
| MMS + $AL_2$ | 48 | 50 | 270 | 2-11 | 5.40±1.50 |
| MMS + $AL_3$ | 48 | 50 | 250 | 1-10 | 5.00±1.50 |
| MMS + $AL_4$ | 48 | 50 | 155 | 1-11 | 4.20±1.50 |
| Control | | | | | |
| Normal | 48 | 50 | 95 | 0-5 | 1.90±1.00 |
| DMSO | 48 | 50 | 94 | 0-5 | 1.88±1.00 |
| DMSO + $AL_2$ | 48 | 50 | 97 | 0-5 | 1.94±1.00 |

AL1-4:Concentrations of alcoholic extract of *A.scholaris*, SCE: Sister chromatid exchange, DMSO: Dimethyl sulfoxide, *A. scholaris: Alstonia scholaris*, SE: Standard error. AL or ALE: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methane sulfonate 5 μg/ml culture. Calculations were made at <0.05 probability

These findings were corroborated by the observation that micronuclei frequency reached the lowest point at 4 mg/ml of the extract. The extract was able to inhibit benzo(a) pyrene-induced mutagenic changes as the frequency of splenocytes bearing one micronucleus and also cells, which bear multiple micronuclei were reduced by the administration of the extract.[26] In our *in vitro* experiments, we observe the enhancement of replication indices that support the above finding.

The anticancer properties of this medicinal plant was evaluated and the tumor incidence, tumor yield, tumor burden and cumulative number of papillomas were found to be higher in the carcinogen treated control compared to animals treated with *A. scholaris* extract. Furthermore, a significant increase in reduced glutathione, superoxide dismutase, and catalase level but decrease in lipid peroxidation was observed in ASE administered experimental groups than the carcinogen with control-treated. This study demonstrated the chemopreventive potential of

**Table 11: Analysis of cell cycle kinetics after treatment with MMS along with alcoholic extracts of *A. scholaris in vitro*, in the absence of $-S_9$ mix**

| Treatment | Cell scored | Cell in (%) | | | RI | 2×3 Chi-square test |
|---|---|---|---|---|---|---|
| | | $M_1$ | $M_2$ | $M_3$ | | |
| MMS | 200 | 60 | 35 | 05 | 1.45 | |
| MMS + $AL_1$ | 200 | 58 | 36 | 06 | 1.48 | Significant |
| MMS + $AL_2$ | 200 | 58 | 37 | 05 | 1.47 | Significant |
| MMS + $AL_3$ | 200 | 55 | 33 | 12 | 1.57 | Significant |
| MMS + $AL_4$ | 200 | 53 | 36 | 11 | 1.58 | Significant |
| Control | | | | | | |
| Normal | 200 | 55 | 30 | 15 | 1.60 | |
| DMSO | 200 | 51 | 35 | 14 | 1.63 | |
| DMSO + $AL_2$ | 200 | 52 | 31 | 17 | 1.65 | |

2×3 Chi-square test were conducted, AL1-4: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methane sulfonate 5 μg/ml culture. Calculations were made at <0.05 probability. DMSO: Dimethyl sulfoxide, *A. scholaris: Alstonia scholaris*, RI: Replication index

**Table 12: Analysis of cell cycle kinetics after treatment with MMS along with alcoholic extracts of *A. scholaris in vitro*, in the presence of $+S_9$ mix**

| Treatment | Cell scored | Cell in (%) | | | RI | 2×3 Chi-square test |
|---|---|---|---|---|---|---|
| | | $M_1$ | $M_2$ | $M_3$ | | |
| MMS | 200 | 59 | 37 | 04 | 1.45 | |
| MMS + $AL_1$ | 200 | 58 | 34 | 08 | 1.50 | Significant |
| MMS + $AL_2$ | 200 | 54 | 35 | 11 | 1.57 | Significant |
| MMS + $AL_3$ | 200 | 52 | 35 | 13 | 1.61 | Significant |
| MMS + $AL_4$ | 200 | 50 | 36 | 14 | 1.64 | Significant |
| Control | | | | | | |
| Normal | 200 | 47 | 37 | 16 | 1.69 | |
| DMSO | 200 | 46 | 38 | 16 | 1.70 | |
| DMSO + $AL_2$ | 200 | 45 | 38 | 17 | 1.72 | |

2×3 Chi-square test were conducted. AL: Concentrations of alcoholic extracts of *A. scholaris*, MMS: Methyl methane sulfonate 5 μg/ml culture. Calculations were made at <0.05 probability. DMSO: Dimethyl sulfoxide, *A. scholaris: Alstonia scholaris*, RI: Replication index

*A. scholaris* bark extract in 7,12-dimethylbenz(a) anthracene-induced skin tumor genesis in albino mice.[27] The aqueous extract at 50 mg/kg bw induced the cellular immune response while at 100 mg/kg bw inhibited the delayed type of hypersensitivity reaction.[7]

An 85% of ethanolic bark extract of *A. scholaris* showed antitumor and radiation sensitizing activity against a mouse transplantable tumor and is cytotoxic to human tumor cell lines.[28] The ethanolic extract of *A. scholaris* was also found to decrease the malondialdehyde level that prevented lipid peroxidation.[29] Other reports also suggested the presence of nitric oxide scavenging activity in case of *A. scholaris*.[9] It was observed the ethanolic extract of *Alternanthera sessilis* is a free radical inhibitor and scavenger acting possibly as a primary antioxidant, an observation, which can be correlated with studies reported by Mau *et al*.[30]

Besides flavonoids and phenolic compounds, some of the alkaloids, saponins and triterpenoids are also reported to possess antioxidant activity.[31] The presence of flavonoids, alkaloids, and triterpenoids in the alcoholic extract of *A. scholaris* has already been reported.[32] The results of the present phytochemical investigation further add to these conclusions.

## CONCLUSION

Alcoholic extracts of *A. scholaris* reduces the total aberrant cells ranges from 10.0% to 41.84% and among them it reduces total frequencies of aberration ranges from 220 to 124 against 290 aberrations causes due to MMS *in vivo*. The same trends were observed in the *in vitro* experiments that is, it reduces CAs 39.62%, 32.83%, and 38.48% ± standard error (SE) at 24, 48, and 72 h of exposure respectively; but when experiments were carried out in the presence of liver $S_9$ fraction, these values were 45.31%, 44.46%, and 38.34% ± SE respectively at <0.05 level, likewise it also reduces SCE from 7.70 to 4.20 ± SE per cell and enhances RI from 1.45 to 1.64.

Alcoholic extracts of *A. scholaris* significantly reduces the number of aberrant cells and frequency of aberration per cell at each concentration and duration of exposure *in vivo;* similarly it reduces CAs and SCE and enhances RI *in vitro* both of which were statistically significant at <0.05 level.

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### Conflicts of interest
There are no conflicts of interest.

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