CHAPTER - 4

ALSTONIA SCHOLARIS
INTRODUCTION

Cancer is among the most dreaded of human diseases. It is considered as an adversary of modernization and the pattern of socio-economical life dominated by western medicine. Cancer still counts as one of the most frequent causes of human fatality, particularly in technically advanced countries. 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 present in medicinal plants commonly used by the human population, which can inhibit the progression of tumor. Cancer chemoprevention is a mean of cancer control by pharmacological intervention of the occurrence of the disease using chemical compounds. Recent development of medical treatment of human disease will be intimately connected to natural products and greater emphasis has been given towards the researches on complementary and alternative medicine that deals with cancer management (Premalatha et al., 2004). Multidisciplinary scientific investigations are making best efforts to combat this disease, but a perfect cure is yet not realized in modern medicine. Many herbs have been evaluated in clinical studies and are currently being investigated phytochemically to understand their antitumour
actions against various cancers. Herbal medicines have a vital role in the prevention and treatment of cancer. Some herbs protect the body from cancer by enhancing detoxification functions of the body (Bradstreet, 1997). Certain biological response modifiers derived from herbs are known to inhibit growth of cancer by modulating the activity of specific hormones and enzymes. Some herbs reduce toxic side effects of chemotherapy and radiotherapy and are often employed for cancer treatment. The traditional Indian system of medicines, Ayurveda, uses about 2,000 plant species, while the Chinese Pharmacopoeia lists over 5,700 traditional medicines, most of which are of plant origin (Nair et al., 2010).

*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, to treat several diseases (Chandra et al., 2003). Common names of *Alstonia scholaris* are chatium, white cheese wood, milky pine, black board tree, devil’s tree, and satni (Chopra et al. 1956).

The plant is grown in the lowland and mountain rainforests of India, the Asia-Pacific, Southern China and Queensland. The plant grows throughout the humid regions of India especially in West Bengal and west-coast forests of south India. The plant is used in Ayurvedic, Unani and
Sidhha types of alternative medicinal systems (Dey, 2011; Joshi SG, 2000). The methanolic extract of this plant was found to exhibit pronounced antiplasmodial activity. The plant is reported to have antimutagenic effect (Arulmozhi et al., 2010).

*Alstonia scholaris* is used to treat chronic skin ulcers, the herb is given to lactating mothers to increase lactation, helps post delivery weakness and digestion, the herb improves the digestive system, and acts as an antipyretic, it is also used for various liver disorders. The herb also benefits in expulsion of worms in stomach. It is used to cure asthma and to cure chronic cough. *Alstonia scholaris* is used in various Ayurvedic preparations like Saptaparnasatvadi vati, Saptachadadi vati, Saptachhadadi vati, Saptacchadadi taila, Saptacchadadi kvatha and Saptaparna ghanasara or uses of *Alstonia scholaris* mainly in Whooping cough, Malaria, Jaundice, Gastric complaint, Headache, Asthma, Stomachache, Wound, Fever.

Ethanolic extract of *Alstonia scholaris* using various in vitro tests including 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical scavenging, metal ion chelating, hydrogen peroxide scavenging, superoxide anion radical scavenging, and ferric thiocyanate reducing ability. Dichloromethane and ethyl acetate fractions were found to have significant
free radical scavenging and metal ion chelating properties (Arulmozhi et al, 2010).

The hepatoprotective effect of *Alstonia scholaris* on liver injuries induced by carbon tetrachloride (CCl4), D-galactosamine, acetaminophen and ethanol were investigated by means of serum-biochemical and histo-pathological examinations. Post treatment of *Alstonia scholaris* reduced dose-dependently the elevation of serum trans-aminases level and histopathological changes such as cell necrosis, inflammatory cell infiltration which were caused by the single administration of 32 l/kg CCl4 or 600 mg/kg acetaminophen in mice. *Alstonia scholaris* Linn significantly lowered 288 mg/kg –D-galactosamine induced serum transaminases elevation in the serum-biochemical analysis in rats. A tendency was also shown to inhibit cell necrosis and inflammatory cell infiltration caused by –D-galactosamine in histopathological examination. All serological and histopathological effects of *Alstonia scholaris* has been reported previously as a treatment criteria of hepatitis (Linn et al, 1996).

The plant is reported to have anti-mutagenic effect (Lim et al, 1990). The bark extract of *Alstonia scholaris* has immune-stimulating effect. The aqueous extract at low dose induced the cellular immune response while at high dose inhibited the delayed type of
hypersensitivity reaction (Iwo et al, 2000). Echitamine chloride, an indole alkaloid, extracted from the bark of *Alstonia scholaris* has promising anticancer effect against sarcoma (Saraswathi et al, 1999; Saraswathi et al, 1998). The plant *Alstonia scholaris* is reported to possess *in vitro* nitric oxide scavenging activity in preliminary studies (Jagetia et al, 2004). The nitric oxide scavenging property of a compound is evidence for free radical and ROS scavenging properties (Lorenz et al, 2003). Several studies have demonstrated that plants produce potent antioxidants and represent important sources of natural antioxidants (Es-Safi et al, 2005; Harish et al, 2006).

The milky juice is applied to ulcers. The most extensively used part of the plant is the bark which is a component of many compound herbal formulations. The bark extract of the powdered stem bark is a bitter tonic and febrifuge, useful for the treatment of malaria, diarrhea, and dysentery. The bark decoction is also used to treat asthma, hypertension, lung cancer, and pneumonia while the leaf infusion is used to treat fever (Channa et al, 2005).

The ethanolic extract of the leaves of *Alstonia scholaris* (30, 300, 1000 and 2000 mg/kg body weight) intraperitoneally did not elicit any
changes in the behavior and autonomic responses of mice compared to controls. None of the mice treated with the up to 2 g/kg of the extract died during the 48 hour observation period following the administration of the extract (Channa et al, 2005). The hydroalcoholic extract of the stem bark of *Alstonia scholaris* showed acute toxic effects that were dependent of the season of collection, the bark collected in the summer was most toxic (LD50 of 900 mg/kg), followed by collection in the winter (LD50 of 1075 mg/kg) while the monsoon season extract yielded the least toxic extract (LD50 of 1200 mg/kg). Administration of the extract by the oral route was less toxic than the intraperitoneal (i.p.) route. Subacute toxicity tests were carried out in rats with daily doses of 120 and 240 mg/kg (corresponding to 1/10th and 1/5th of the LD50 dose) for 30 days. No changes were seen in general behaviour, physiological activities, or final body weights of the rats receiving the lower dose and none of these rats died. The higher dose caused lethargy in the rats.

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. Additionally, the phytochemicals like echitamine, alstonine, pleiocarpamine, O-
methylmacralstonine, macralstonine, and lupeol are also reported to possess antineoplastic effects. In addition to the cytotoxic effects, *Alstonia scholaris* is also observed to possess radiomodulatory, chemomodulatory, and chemopreventive effects and free-radical scavenging, antioxidant, anti-inflammatory, antimitagenic, and immunomodulatory activities, all of which are properties efficacious in the treatment and prevention of cancer (Baliga MS, 2010).

### RESULTS

**IN VIVO EFFECTS:**

In this study the Albino mice were exposed till 16h of treatments and found that the percent aberrant cells were 8.2, 8.0, 7.8, 7.3 and 6.8 of five different concentrations of *Alstonia* extract respectively against 9.8% of aberrant cells induced by Methylmethanesulphonate (MMS). 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 values were 16.32, 18.36, 20.40, 25.51, and 30.61 against five different concentrations of *Alstonia* extract respectively. The maximum effect of *Alstonia* extract was
30.61% of the $V^{th}$ concentration of Alstonia extract (Table 4.1, Fig 11).

The effect on the total number of frequencies per thousand cells was 164, 155, 146, 135, and 136 of five consecutive concentrations of Alstonia extract against 176 of MMS alone. The normal values were 20 for distilled water treatment and 23 and 19 for DMSO and Alstonia extract alone treatment (Table 4.2). When the treatment durations were increase to 24h, still the effects were following same trend, with increasing values. The observed values were 11.5, 11.0, 10.3, 9.5 and 8.7 percent of five concentrations of Alstonia extract respectively against 13.5 percent of MMS alone. The values of normal were 1.9, 2.2 & 2.1 percent for pure water, DMSO and Alstonia extract alone respectively. Only $V^{th}$ concentration shows a noticeable effect on the percentage reductions of aberrant cell (Table 4.3 Fig 12).

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 cells were 182, 175, 173, 156, and 150 for Alstonia extract along with MMS alone (Table 4.4). At 32h exposure, the percent aberrant cells observed were 15.0 percent for MMS alone, and 13.0, 12.2, 11.2, 10.4, 9.0 percent for five different concentrations of Alstonia extract along with MMS, whereas the values for normal control was 1.8 and DMSO
& Alstonia extract alone treatment were 2.3 and 2.0 percent respectively. In terms of the effects on the percent reduction in aberrant cells, the values were 13.33, 18.66, 25.33, 30.66 and 40.00 percent respectively. These values show 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 4.5 and Fig 13).

The total aberrant chromosomal frequencies per thousand cells were 236 for MMS and 196, 193, 180, 171, 150 for Alstonia extract along with MMS of five different concentrations of Alstonia extract. These frequencies, with significant reduced value, show the effects of Alstonia extract on the total aberrations as well as aberrations per cell as shown in (Table 4.6).

**IN VITRO EFFECTS:**

Treatment with MMS results in clastogenic abnormalities as observed in percent metaphase aberration, types of aberrations and aberration per cell were 39.75, 67.00, 69.50 percent or 0.40, 0.67 & 0.70 aberration per cell, whereas control the normal & DMSO plus Alstonia extract 04.00, 04.50 per cell at single standard dosage and three various durations viz, 24, 48 and 72
Alstonia extract bring down aberrations from 39.75% to 32.50, 28.75, 26.25 and 24.00 percent with four consecutive dosages of Alstonia extract at 24 h of duration, whereas at 48 h, it lower from 67.00% to 50.50, 43.35, 49.00, and 45.00 percent by 1st to 4th concentrations of Alstonia extract respectively. Same trend were noticed, when the treatment durations was increased to 72 h. These values show linear increasing trend with dosages, but it does not dependant on durations. The maximum percentage reductions in the aberrations were 39.62 for 24 h and 32.83 & 38.48 for 48 and 72 h respectively (Table 4.7, Fig 14).

When culture was setup along with metabolic activation system (+S9 mix), the effect of MMS increased. Similarly the effects of Alstonia extract also lower the clastogenic activity of MMS. These values show linearly increasing trend with doses (Table 4.8 Fig 15). The maximum effective percentage reductions were 45.31, 44.46, and 38.34 percent for 24, 48 and 72 h respectively. The highest reduction on clastogeny of cells was noticed at 24h durations; though the other values were also statistically significant.

In another marker the experiment were conducted and sister chromatid exchanges were counted (Table 4.9, 4.10), 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 the analysis of SCE, only 48 h of cultures were used and 50 metaphases were scored.

The effects of Alstonia extract on replication index (Table 4.11, 4.12) show on elevated level when compared from the MMS treatment i.e. from 1.44 to 1.68. Though lower than the normal level of 1.71. The effect, after treatment with metabolic activation system shows from 1.43 to 1.66 i.e., much effective than without metabolic activation system. Therefore, we observed that Alstonia extract has potent anti-clastogenic activities in these experiments.
In Vivo Effect of Alstonia extract at 16 hrs

% Reduction of Castogeny

Doses of Alstonia extract

AL1: 16.32
AL2: 18.36
AL3: 20.4
AL4: 25.51
AL5: 30.61

Fig- 12
Fig- 13

In Vivo Effect of Alstonia extract at 24 hrs

% Reduction of Clastogenicity

Doses of Alstonia extract

AL1  AL2  AL3  AL4  AL5

14.81  18.51  23.7  29.62  35.55
Fig- 14

In vitro effect of Alstonia extract (-S9)

% Clastogenicity

Concentration of Alstonia extract

N MMS AL1 AL2 AL3 AL4

24 48 72
Fig-15

In vitro effect of Alstonia extract (+S9)

%Clastogeny

Concentration of Alstonia extract
**DISCUSSION**

Antioxidants also play an important role in cancer prevention. Cancer cells are "immortal" i.e. 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 by the action of antioxidants. In our experiment the protective effects of Alastonia extract may be due to this reason i.e quenching the free radicals that were generated due to mutagen and carcinogen.

The real source for antioxidant is a plant food. There are many different compounds with in fruits, vegetables, nuts, seeds, legumes, and grains that exert antioxidant activity. This is one of the reasons that plant based diets are associated with good health, including reduced risk to breast cancer.

Today, nearly 25% of all prescriptions contain compounds that come directly or indirectly from plants. Now days, much importance is given to discover more plants with anticancer effect. The disadvantages of chemotherapy and Immunotherapy in the treatment of cancer have paved the
way for herbal treatment for cancer. The advantages of herbal treatment are no side effects, less cost and relapse of the disease is low.

The anticancer effect of various doses of an alkaloid fraction of *Alstonia scholaris* (ASERS), was studied in vitro in cultured human neoplastic cell lines (HeLa, HepG2, HL60, KB and MCF-7) and in Ehrlich ascite carcinoma bearing mice. 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. However, exposure of cells to ASERS for 4h 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. Treatment of various cells with ASERS resulted in a concentration dependent decline in the viable cells and a nadir was reached at 200g/mL in the entire cell lines studied, we too have notices similar trend in our *in vitro* experiment. The IC50 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 9 consecutive days to the tumor bearing mice caused a dose dependent remission of the tumor up to 240 mg/kg body weight, 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 post-tumor-cell inoculation as against no survivors in the saline treated control group (Jagetia et al, 2006).

The chemomodulatory activity of *Alstonia scholaris* extract studied in combination with berberine hydrochloride (BCL), a topoisomerase inhibitor, in Ehrlich ascites carcinoma-bearing mice. The tumor-bearing animals were injected with various doses of ASE and 8 mg/kg of BCL (one-fifth of the 50% lethal dose) was combined with different doses of ASE (60-240 mg/kg). The combination of 180mg/kg of ASE with 8 mg/kg of BCL showed the greatest antitumor effect; the number of tumor-free survivors was more, and the medium survival time and the average survival time increased up to 47 and 40.5 days, respectively, when compared with either treatment alone.

Similarly, when 180 mg/kg of ASE was combined with different doses of BCL (2-12 mg/kg), a dose-dependent increase in the anticancer activity was observed up to 8 mg/kg of BCL. However, a further increase in the BCL dose to 10 and 12 mg/kg resulted in toxic side effects. The best effect was observed when 180 mg/kg of ASE was combined with 6 or 8 mg/kg of BCL, where an increase in the antineoplastic activity was reported (Jagetia et al, 2004).
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) anthrecene (100lg/100 ll acetone), and two weeks later, promoted by repeated application of croton oil (1% in incidence, tumor yield, tumor burden and cumulative number of papillomaas were 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 but decrease in lipid peroxidation was measured in ASE administered experimental groups than the carcinogen treated control (Jahan et al, 2009).

Chemopreventive agents can be targeted by intervention at the initiation, promotion, or progression stage of multistage carcinogenesis (Wattenberg, 1990; Kelloff et al, 1994; Morse and Stoner, 1993; Stoner and Mukhtar, 1995). The intervention of cancer at the promotion stage, however, seems to be the most appropriate and practical. The major reason for that relates to the fact that tumor promotion is reversible event at least in early stages and requires repeated and prolonged exposure of a promoting agent (Di Giovanni, 1992).
The cytotoxicity of different fractions of A. scholaris in HeLa cells was dependent on the season of collection be it, the monsoon, winter or summer (Jagetia et al, 2005). The most toxic extract was obtained from the bark collected in the summer followed by that collected in the winter and the least toxic was when collection was in the monsoon season. Cytotoxicity was highest with the residue fraction and lowest for the steroidal fraction. A major alkaloid, alstonine has antitumor activity in YC8 lymphoma and Ehrlich ascites carcinoma cells (Jagetia et al, 2005). Bisindole and villalstonine showed marked activity against human cancer cell lines, MOR-P (adenocarcinoma), COR-L23 (large cell carcinoma) cell lines although in these cells, pleiocarpamine, Omethylmacralstonine and macralstonine were much less active than villalstonine. Oacetylmacralstonine, villalstonine and macrocarpamine were cytotoxic to human cancer cell lines, MOR-P, COR-L23 StMI1 1a (melanoma), Caki-2 (renal cell carcinoma), MCF7 (breast adenocarcinoma) and LS174T (colon adenocarcinoma) (Jagetia et al, 2005).

The hydroalcoholic extract of A. scholaris protected against benzo(a)pyrene-induced for stomach carcinoma in female mice when added to drinking water at doses of 1, 2 and 4 mg/mL for 2 weeks before, during and 2 weeks after the carcinogen exposure. These doses reduced tumour multiplicity by 21.43, 28.57 and 50%, respectively. The greatest protection
was afforded by the highest dose which reduced tumour incidence by 6.67% (Jagetia et al, 2003). Tumour multiplicity incidence was significantly reduced (91.93% with extract versus 100% in benzo(a)pyrene-treated mice) by 4 mg/mL dose that was added to the drinking water during the post-initiation period, starting at 48 h after the last dose of benzo(a)pyrene (post-treatment) and continued for 8 weeks. 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 micronuclei and also cells which bear multiple micronuclei were reduced by the extract (Jagetia et al, 2003). In our in vitro experiment, we observe the enhancement of replication indices that supported 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 compred to animals treated with Alstonia scholaris extract. Furthermore, a significant increase in reduced glutathione, superoxide dismutase and catalase but decrease in lipid peroxidation was measured in ASE administered experimental groups than the carcinogen treated control. This study demonstrated the chemopreventive potential of Alstonia scholaris bark
extract in DMBA- induced skin tumor genesis in Albino mice (Swafiya et al, 2010). The aqueous extract at 50mg/kg b.w. induced the cellular immune response while at 100mg/kg b.w. inhibited the delayed type of hypersensitivity reaction, (Maria et al, 2000).

An 85% ethanolic bark extract of A. scholaris showed antitumor and radiation sensitising activity against a mouse transplantable tumor and is cytotoxic to human tumour cell lines (Baliga et al, 2004). The ethanolic extract of Alstonia scholaris was found to decrease the malondialdehyde level and prevented lipid peroxidation (Arulmozhi et al, 2007). Reports suggested presence of nitric oxide scavenging activity in Alstonia scholaris (Jagetia et al, 2004).

It was observed the EEAS is free radical inhibitor or scavenger acting possibly as primary antioxidants, which can be correlated with previous studies reported by Jeng-Leun Mau (2001).

H$_2$O$_2$ is highly important because of its ability to penetrate biological membranes. H$_2$O$_2$ itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. The results showed that EEAS had an effective H$_2$O$_2$ scavenging activity. Superoxide anion radicals are produced endogenously by flavoenzymes like xanthine oxidase, which converts hypoxanthine to xanthine and subsequently to uric
acid in ischemia-reperfusion (Ilhami et al, 2005). The decrease of absorbance at 560 nm with EEAS and antioxidants indicates the consumption of superoxide anion in the reaction mixture.

In addition to flavonoids and phenolic compounds, some of the alkaloids, saponins and triterpenoids are reported to possess antioxidant activity (Sujay et al, 2006). The presence of flavonoids, alkaloids and triterpenoids in alcoholic extract of *Alstonia scholaris* has been reported (Khan et al, 2003) and the results of preliminary phytochemical investigation in the present study also further substantiates this. Hence, the observed *in vitro* antioxidant activity may be because of these phytoconstituents, which needs further investigation.
**TABLE 4.1** Chromosome aberrations induced by Methylmethanesulphonate (MMS x/kg. bw) at 16 h of treatment with the effect of *Alstonia scholaris* on the frequency of cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AS (Y/Kg. bw)</th>
<th>Cell with Pulverized Chromosome</th>
<th>Types of chromatic aberrations</th>
<th>Aberrant cell No. (%)</th>
<th>(% Red)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH₂O</td>
<td>0</td>
<td>00</td>
<td>02 03 15 00</td>
<td>18 (1.8)</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
<td>00</td>
<td>03 01 19 00</td>
<td>20 (2.0)</td>
<td></td>
</tr>
<tr>
<td>MMS</td>
<td>0</td>
<td>17</td>
<td>52 28 43 05</td>
<td>98 (9.8)</td>
<td></td>
</tr>
<tr>
<td>AS Extract</td>
<td>AS₅</td>
<td>00</td>
<td>05 02 15 00</td>
<td>17 (1.7)</td>
<td></td>
</tr>
<tr>
<td>MMS+ AS Extract</td>
<td>AS₁</td>
<td>13</td>
<td>51 30 31 04</td>
<td>82 (8.2)</td>
<td>16.32</td>
</tr>
<tr>
<td></td>
<td>AS₂</td>
<td>12</td>
<td>48 28 32 04</td>
<td>80 (8.0)</td>
<td>18.36*</td>
</tr>
<tr>
<td></td>
<td>AS₃</td>
<td>10</td>
<td>43 31 31 03</td>
<td>78 (7.8)</td>
<td>20.40*</td>
</tr>
</tbody>
</table>
### Note:
AS; concentrations of extracts of *Alstonia scholaris*, Methylmethanesulphonate 5 μg/ml /kg body weight) at 16 h of treatment. Calculations were made excluding the gaps type of aberration and *significant at < 0.05 probability. AS/kg.bw is the concentration of extracts of *Alstonia scholaris*.

#### TABLE 4.2

Chromosome aberrations induced by MMS (MMS x/kg bw) with the effect of *Alstonia scholaris* extract on the total number and types of frequency of cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AS (Y/Kg. bw)</th>
<th>Cell with aberration</th>
<th>Total number of Aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH₂O</td>
<td>0</td>
<td>982 16 02 00 00 00</td>
<td>20</td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
<td>980 17 03 00 00 00</td>
<td>23</td>
</tr>
<tr>
<td>MMS</td>
<td>0</td>
<td>902 60 22 05 04 03</td>
<td>176</td>
</tr>
<tr>
<td>AS Extract</td>
<td>AS₅</td>
<td>983 15 02 00 00 00</td>
<td>19</td>
</tr>
<tr>
<td>MMS +AS Extract</td>
<td>AS₁</td>
<td>918 47 19 04 03 04</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>AS₂</td>
<td>920 47 18 03 05 04</td>
<td>155*</td>
</tr>
</tbody>
</table>
Note: AS; concentrations of extracts of *Alstonia scholaris*, Methylmethanesulphonate 5 μg/ml /kg body weight) at 16 h of treatment. Calculations were made excluding the gaps type of aberration and *significant at <0.05 probability. AS/kg.bw is the concentration of *Alstonia scholaris* extracts. The animals were sacrificed 16 h after MMS treatment 1000 cells from 10 animals were analyzed for each point.

<p>| | | | | | | | |</p>
<table>
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<tbody>
<tr>
<td>AS3</td>
<td>922</td>
<td>49</td>
<td>16</td>
<td>02</td>
<td>04</td>
<td>03</td>
<td>04</td>
</tr>
<tr>
<td>AS4</td>
<td>927</td>
<td>45</td>
<td>15</td>
<td>04</td>
<td>03</td>
<td>03</td>
<td>135*</td>
</tr>
<tr>
<td>AS5</td>
<td>932</td>
<td>39</td>
<td>14</td>
<td>04</td>
<td>04</td>
<td>03</td>
<td>136*</td>
</tr>
</tbody>
</table>

**TABLE 4.3** Chromosome aberrations induced by Methylmethane-sulphonate (MMS x/kg. bw) at 24 h of treatment with the effect of *Alstonia scholaris* on the frequency of cells.
### Types of chromatic aberrations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AS (Y/Kg. bw)</th>
<th>Cell with Pulverized Chromosome</th>
<th>Types of chromatic aberrations</th>
<th>Aberrant cell No. (%)</th>
<th>(%) Red</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gap Break Fragement Exchange</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DH₂O</td>
<td>0 00</td>
<td>01 01 18 00</td>
<td>19 (1.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>0 00</td>
<td>02 03 19 00</td>
<td>22 (2.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMS</td>
<td>0 22</td>
<td>56 54 55 02</td>
<td>135 (13.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS Extract</td>
<td>AS₅</td>
<td>00 03 17 00</td>
<td>21 (2.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMS+ AS Extract</td>
<td>AS₁</td>
<td>09 49 36 60 05</td>
<td>115 (11.5) 14.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AS₂</td>
<td>08 52 38 56 04</td>
<td>110 (11.0) 18.51*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AS₃</td>
<td>09 47 38 50 03</td>
<td>103 10.3 23.70*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AS₄</td>
<td>07 41 40 48 00</td>
<td>95 (9.5) 29.62*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AS₅</td>
<td>07 36 39 41 00</td>
<td>87 (8.7) 35.55*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** AS; concentrations of extracts of *Alstonia scholaris*, Methylmethanesulphonate 5 μg/ml/kg body weight) at 24 h of treatment. Calculations were made excluding the gaps type of aberration and *significant at < 0.05 probability. AS/kg.bw is the concentration of extracts of *Alstonia scholaris*.

**TABLE 4.4** Chromosome aberrations induced by MMS (MMS x/kg bw) with the effect of *Alstonia scholaris* extract on the total number and types of frequency of cells.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>AS (Y/Kg. bw)</th>
<th>Cell with aberration</th>
<th>Total number of Aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH₂O</td>
<td>0</td>
<td>981 17 02 00 00 00</td>
<td>21</td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
<td>978 20 02 00 00 00</td>
<td>24</td>
</tr>
<tr>
<td>MMS</td>
<td>0</td>
<td>865 103 15 07 05 02 03</td>
<td>205</td>
</tr>
<tr>
<td>AS Extract</td>
<td>AS₅</td>
<td>979 19 02 00 00 00</td>
<td>23</td>
</tr>
<tr>
<td>MMS + AS Extract</td>
<td>AS₁</td>
<td>885 86 14 05 03 04 03</td>
<td>182</td>
</tr>
<tr>
<td></td>
<td>AS₂</td>
<td>890 83 13 04 04 02 04</td>
<td>175*</td>
</tr>
<tr>
<td></td>
<td>AS₃</td>
<td>897 73 14 04 03 04 04</td>
<td>173*</td>
</tr>
<tr>
<td></td>
<td>AS₄</td>
<td>905 68 13 05 04 02 03</td>
<td>156*</td>
</tr>
<tr>
<td></td>
<td>AS₅</td>
<td>913 63 10 04 03 03 04</td>
<td>150*</td>
</tr>
</tbody>
</table>

**Note:** AS; concentrations of extracts of *Alstonia scholaris*, Methylmethylene-sulphonate 5 μg/ml /kg body weight) at 24 h of treatment. Calculations were made excluding the gaps type of aberration and *significant at <0.05 probability. AS/kg.bw is the concentration of *Alstonia scholaris* extracts. The animals were sacrificed 24 h after MMS treatment 1000 cells from 10 animals were analyzed for each point.

**TABLE 4.5** Chromosome aberrations induced by Methylmethylene-sulphonate (MMS x/kg. bw) at 32 h of treatment with the effect of *Alstonia scholaris* on the frequency of cells.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>AS (Y/Kg. bw)</th>
<th>Cell with Pulverized Chromosomes</th>
<th>Types of chromatic aberrations</th>
<th>Aberrant cell No. (%)</th>
<th>(%) Red</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gap</td>
<td>Break</td>
<td>Fragment</td>
</tr>
<tr>
<td>DH₂O</td>
<td>0</td>
<td>0</td>
<td>01</td>
<td>01</td>
<td>17</td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
<td>0</td>
<td>02</td>
<td>02</td>
<td>21</td>
</tr>
<tr>
<td>MMS</td>
<td>0</td>
<td>25</td>
<td>42</td>
<td>27</td>
<td>90</td>
</tr>
<tr>
<td>AS Ext</td>
<td>AS₅</td>
<td>0</td>
<td>05</td>
<td>02</td>
<td>18</td>
</tr>
<tr>
<td>MMS+ AS Extract</td>
<td>AS₁</td>
<td>15</td>
<td>40</td>
<td>29</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>AS₂</td>
<td>13</td>
<td>38</td>
<td>28</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>AS₃</td>
<td>11</td>
<td>36</td>
<td>25</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>AS₄</td>
<td>09</td>
<td>34</td>
<td>21</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>AS₅</td>
<td>09</td>
<td>35</td>
<td>14</td>
<td>61</td>
</tr>
</tbody>
</table>

**Note:** AS; concentrations of extracts of *Alstonia scholaris*, Methylmethanesulphonate 5 μg/ml /kg body weight) at 32 h of treatment. Calculations were made excluding the gaps type of aberration and *significant at < 0.05 probability. AS/kg.bw is the concentration of extracts of *Alstonia scholaris*. 
TABLE 4.6 Chromosome aberrations induced by MMS (MMS x/kg bw) with the effect of *Alstonia scholaris* extract on the total number and types of frequency of cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AS (Y/Kg. bw)</th>
<th>Cell with aberration</th>
<th>Total number of Aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DH₂O</td>
<td>0</td>
<td>982</td>
<td>16</td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
<td>977</td>
<td>22</td>
</tr>
<tr>
<td>MMS</td>
<td>0</td>
<td>850</td>
<td>107</td>
</tr>
<tr>
<td>AS Extract</td>
<td>AS₅</td>
<td>980</td>
<td>18</td>
</tr>
<tr>
<td>MMS +AS Extract</td>
<td>AS₁</td>
<td>870</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>AS₂</td>
<td>888</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>AS₃</td>
<td>888</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>AS₄</td>
<td>896</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>AS₅</td>
<td>910</td>
<td>63</td>
</tr>
</tbody>
</table>

**Note:** AS; concentrations of extracts of *Alstonia scholaris*, Methylmethanesulphonate 5 μg/ml /kg body weight) at 32 h of treatment. Calculations were made excluding the gaps type of aberration and *significant at <0.05 probability. AS/kg.bw is the concentration of *Alstonia*
scheiarls extracts. The animals were sacrificed 32 h after MMS treatment 1000 cells from 10 animals were analyzed for each point.

**TABLE 4.7** Analysis of Chromosomal aberrations after treatment with Methylmethanesulphonate along with *Alstonia scholaris* extract *in vitro* in the absence of -S₉ mix.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Durations (h)</th>
<th>Metaphase scored</th>
<th>Percent aberration metaphase</th>
<th>Types of Aberration (%)</th>
<th>Aberration/Cell ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Include gap</td>
<td>Excluding gap</td>
<td>Chromatid</td>
</tr>
<tr>
<td>MMS</td>
<td>24</td>
<td>200</td>
<td>25.00</td>
<td>23.50</td>
<td>27.50</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>200</td>
<td>40.25</td>
<td>36.25</td>
<td>43.75</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200</td>
<td>42.75</td>
<td>37.35</td>
<td>48.25</td>
</tr>
<tr>
<td>MMS + AS₁</td>
<td>24</td>
<td>200</td>
<td>20.70</td>
<td>15.00</td>
<td>21.00</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>200</td>
<td>30.25</td>
<td>27.50</td>
<td>33.25</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200</td>
<td>33.50</td>
<td>29.70</td>
<td>36.50</td>
</tr>
<tr>
<td>MMS + AS₂</td>
<td>24</td>
<td>200</td>
<td>16.50</td>
<td>14.25</td>
<td>18.50</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>200</td>
<td>26.75</td>
<td>24.25</td>
<td>28.00</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200</td>
<td>30.00</td>
<td>27.50</td>
<td>33.25</td>
</tr>
<tr>
<td>MMS + AS₃</td>
<td>24</td>
<td>200</td>
<td>15.75</td>
<td>14.00</td>
<td>16.50</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>200</td>
<td>24.20</td>
<td>22.30</td>
<td>24.25</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200</td>
<td>28.00</td>
<td>24.50</td>
<td>31.50</td>
</tr>
<tr>
<td>MMS + AS₄</td>
<td>24</td>
<td>200</td>
<td>14.50</td>
<td>13.50</td>
<td>14.50</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>200</td>
<td>23.00</td>
<td>21.50</td>
<td>22.25</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200</td>
<td>26.50</td>
<td>22.75</td>
<td>28.50</td>
</tr>
<tr>
<td>Control</td>
<td>72</td>
<td>200</td>
<td>3.50</td>
<td>1.50</td>
<td>2.50</td>
</tr>
</tbody>
</table>
Note: AS; concentrations of *Alstonia scholaris* extracts, MMS x/kg.bw; Methylmethanesulphonate 5 μg/ml/culture, gaps type of aberration is not included, SE; Standard error, DMSO; dimethyl sulphoxide. Calculations were significant at < 0.05 probability level.

### TABLE 4.8

Analysis of Chromosomal aberrations after treatment with Methylmethanesulphonate along with *Alstonia scholaris* extract *in vitro* in the presence of +S₉ mix.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Durations (h)</th>
<th>Metaphase scored</th>
<th>Percent aberration metaphase</th>
<th>Types of Aberration (%)</th>
<th>Aberration/Cell ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Include gap</td>
<td>Excluding gap</td>
<td>Chromatid</td>
</tr>
<tr>
<td>MMS</td>
<td>24</td>
<td>200</td>
<td>22.00</td>
<td>19.00</td>
<td>23.50</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>200</td>
<td>37.50</td>
<td>32.25</td>
<td>40.00</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200</td>
<td>39.25</td>
<td>33.00</td>
<td>45.25</td>
</tr>
<tr>
<td>MMS+AS₁</td>
<td>24</td>
<td>200</td>
<td>15.50</td>
<td>12.75</td>
<td>18.25</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>200</td>
<td>27.75</td>
<td>25.25</td>
<td>31.50</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200</td>
<td>30.25</td>
<td>27.50</td>
<td>35.00</td>
</tr>
<tr>
<td>MMS+AS₂</td>
<td>24</td>
<td>200</td>
<td>14.25</td>
<td>12.50</td>
<td>16.50</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>200</td>
<td>23.50</td>
<td>22.25</td>
<td>25.50</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200</td>
<td>26.25</td>
<td>23.50</td>
<td>31.25</td>
</tr>
<tr>
<td>MMS+AS₃</td>
<td>24</td>
<td>200</td>
<td>14.50</td>
<td>13.25</td>
<td>14.25</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>200</td>
<td>22.50</td>
<td>21.35</td>
<td>21.50</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>200</td>
<td>25.75</td>
<td>21.50</td>
<td>30.25</td>
</tr>
<tr>
<td>MMS+AS₄</td>
<td>24</td>
<td>200</td>
<td>13.75</td>
<td>12.35</td>
<td>11.50</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>200</td>
<td>21.50</td>
<td>20.00</td>
<td>22.35</td>
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<td></td>
<td>72</td>
<td>200</td>
<td>24.50</td>
<td>19.75</td>
<td>27.50</td>
</tr>
<tr>
<td>Control</td>
<td>72</td>
<td>200</td>
<td>2.30</td>
<td>1.80</td>
<td>1.75</td>
</tr>
<tr>
<td>DMSO+AS₂</td>
<td>72</td>
<td>200</td>
<td>3.50</td>
<td>1.50</td>
<td>2.00</td>
</tr>
</tbody>
</table>
Note: AS; concentrations of *Alstonia scholaris* extracts, MMS x/kg.bw; Methylmethanesulphonate 5 μg/ml/culture, gaps type of aberration is not included, SE; Standard error, DMSO; dimethyl sulphoxide. Calculations were significant at < 0.05 probability level.

**TABLE 4.9** Analysis of sister chromatid exchange after treatment with Methylmethanesulphonate along with *Alstonia scholaris* extract *in vitro*, in the absence of -S₉ mix.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration (h)</th>
<th>METAPHASE SCORED</th>
<th>Total</th>
<th>Range</th>
<th>SCE/Cell ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMS</td>
<td>48</td>
<td>50</td>
<td>385</td>
<td>1 — 11</td>
<td>7.70 ± 1.50</td>
</tr>
<tr>
<td>MMS + AS₁</td>
<td>48</td>
<td>50</td>
<td>330</td>
<td>1 — 11</td>
<td>6.60 ± 1.50</td>
</tr>
<tr>
<td>MMS + AS₂</td>
<td>48</td>
<td>50</td>
<td>275</td>
<td>1 — 10</td>
<td>5.50 ± 1.50</td>
</tr>
<tr>
<td>MMS + AS₃</td>
<td>48</td>
<td>50</td>
<td>245</td>
<td>1 — 10</td>
<td>4.90 ± 1.50</td>
</tr>
<tr>
<td>MMS + AS₄</td>
<td>48</td>
<td>50</td>
<td>215</td>
<td>1 — 10</td>
<td>4.30 ± 1.50</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>48</td>
<td>50</td>
<td>91</td>
<td>0 — 4</td>
<td>1.82 ± 1.00</td>
</tr>
<tr>
<td>DMSO</td>
<td>48</td>
<td>50</td>
<td>94</td>
<td>0 — 5</td>
<td>1.88 ± 1.00</td>
</tr>
<tr>
<td>DMSO+AS₂</td>
<td>48</td>
<td>50</td>
<td>90</td>
<td>0 — 4</td>
<td>1.80 ± 1.00</td>
</tr>
</tbody>
</table>

Note: AS; concentrations of *Alstonia scholaris* extracts, MMSx/kg.bw; Methylmethanesulphonate 5 μg/ml/culture, SE; Standard error, DMSO; dimethyl sulphoxide. Calculations were significant at < 0.05 probability level.
TABLE 4.10 Analysis of Sister chromatid exchange after treatment with Methylmethanesulphonate along with *Alstonia scholaris* extract *in vitro*, in the presence of +S₉ mix.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration (h)</th>
<th>METAPHASE SCORED</th>
<th>Total</th>
<th>Range</th>
<th>SCE /Cell ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMS</td>
<td>48</td>
<td>50</td>
<td>360</td>
<td>3 — 12</td>
<td>7.20 ±1.50</td>
</tr>
<tr>
<td>MMS + AS₁</td>
<td>48</td>
<td>50</td>
<td>310</td>
<td>1 — 11</td>
<td>6.20 ±1.50</td>
</tr>
<tr>
<td>MMS + AS₂</td>
<td>48</td>
<td>50</td>
<td>270</td>
<td>2 — 11</td>
<td>5.40 ± 1.50</td>
</tr>
<tr>
<td>MMS + AS₃</td>
<td>48</td>
<td>50</td>
<td>250</td>
<td>1 — 10</td>
<td>5.00 ± 1.50</td>
</tr>
<tr>
<td>MMS + AS₄</td>
<td>48</td>
<td>50</td>
<td>155</td>
<td>1 — 11</td>
<td>4.20 ± 1.50</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>48</td>
<td>50</td>
<td>95</td>
<td>0 — 5</td>
<td>1.90 ± 1.00</td>
</tr>
<tr>
<td>DMSO</td>
<td>48</td>
<td>50</td>
<td>94</td>
<td>0 — 5</td>
<td>1.88 ± 1.00</td>
</tr>
<tr>
<td>DMSO + AS₂</td>
<td>48</td>
<td>50</td>
<td>97</td>
<td>0 — 5</td>
<td>1.94 ± 1.00</td>
</tr>
</tbody>
</table>

Note: AS; concentrations of *Alstonia scholaris* extracts, MMS x/kg.bw; Methylmethanesulphonate 5 μg/ml/culture, SE; Standard error, DMSO; dimethyl sulphoxide. Calculations were significant at < 0.05 probability level.
TABLE 4.11 Analysis of cell cycle kinetics after treatment with Methylmethanesulphonate along with *Alstonia scholaris* extract *in vitro*, in the absence of -S₉mix.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell scored</th>
<th>(% cell in M₁, M₂, M₃)</th>
<th>Replication Index</th>
<th>2×3 chi square Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMS</td>
<td>200</td>
<td>61 34 05</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>MMS + AS₁</td>
<td>200</td>
<td>58 36 06</td>
<td>1.48</td>
<td></td>
</tr>
<tr>
<td>MMS + AS₂</td>
<td>200</td>
<td>54 35 11</td>
<td>1.57 Significant</td>
<td></td>
</tr>
<tr>
<td>MMS + AS₃</td>
<td>200</td>
<td>50 38 12</td>
<td>1.62 Significant</td>
<td></td>
</tr>
<tr>
<td>MMS + AS₄</td>
<td>200</td>
<td>49 40 13</td>
<td>1.68 Significant</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>200</td>
<td>44 41 15</td>
<td>1.71</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>200</td>
<td>41 45 14</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>DMSO + AS₂</td>
<td>200</td>
<td>38 46 16</td>
<td>1.78</td>
<td></td>
</tr>
</tbody>
</table>

Note: 2×3 Chi square ($\chi^2$) test were conducted, AE; concentrations *Alstonia scholaris* extracts, MMS x/kg.bw; Methylmethanesulphonate 5 μg/ml/ culture, DMSO; dimethyl sulphoxide. Calculations were made at < 0.05 probability level.
**TABLE 4.12** Analysis of cell cycle kinetics after treatment with Methylmethanesulphonate along with *Alstonia scholaris* extract, *in vitro*, in the presence of +S₉mix.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell scored</th>
<th>(% cell in</th>
<th>Replication Index</th>
<th>2×3 chi square Test</th>
</tr>
</thead>
<tbody>
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Note: 2×3 Chi square (χ²) test were conducted, AE; concentrations *Alstonia scholaris* extracts, MMS x/kg.bw; Methylmethanesulphonate 5 μg/ml/culture, DMSO; dimethyl sulfoxide. Calculations were made at < 0.05 probability level.
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