Antigenotoxic and Antimutagenic Effects of *Andrographis paniculata*, a Traditional Medicinal Herb against Genotoxicity of Cyclophosphamide: An *In Vitro* Study on Human Peripheral Lymphocytes

Ayyakkannu Purushothaman¹, Parveen Sufiya¹, Packirisamy Meenatchi¹, Ramalingam Sundaram¹, and Nallappan Saravanan²

¹Post-Graduate and Research Department of Biochemistry, Mohamed Sathak College of Arts and Science, University of Madras, Chennai, Tamil Nadu 600119, India
²Department of Zoology, Government Arts College, Periyar University, Salem, Tamil Nadu 636007, India

**ABSTRACT:** *Andrographis paniculata* (family: Acanthaceae) is a medicinal herb used in Indian system of medicine (Ayurveda, Siddha, and Unani), traditional and folk systems to treat various illnesses. This study examined the phytochemical constituents of ethanol extract from *A. paniculata* and its protective effect against genotoxicity caused by cyclophosphamide (CPA). Phytochemical screening and estimation of total phenolic content were analyzed using standard methods. The bioactive components from the ethanol extract of *A. paniculata* (EAP) were analyzed using gas chromatography-mass spectrometry. To investigate the protective effect of EAP against CPA-induced genotoxicity, human peripheral lymphocyte cultures were used. To test the antigenotoxic and antimutagenic effects of EAP, lymphocytes were treated with different concentrations (50–250 mg/mL) alone and co-treated along with CPA+EAP for 48 h. The cells were analyzed for structural chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) in control, CPA treated, and CPA+EAP co-treated lymphocytes. Results of the study revealed that the lymphocyte cultures which had 48 h continuous exposure to EAP (50–250 mg/mL) alone did not show any significant changes in CAs and SCE frequencies. These results substantiate the antimutagenic nature of the extract. Furthermore, the lymphocytes co-treated with CPA along with extract showed a significant reduction in CAs (reduced from 26.50±2.50% to 11.00±1.00%) and SCEs (reduced from 9.92±0.63 per cell to 4.56±0.18 per cell). These results suggest that *A. paniculata* is protective against CPA induced genotoxicity and put forward its possible use as a supplement with chemotherapeutic drugs.

**Keywords:** *Andrographis paniculata*, chromosomal aberrations, cyclophosphamide, genotoxicity, sister chromatid exchange

**INTRODUCTION**

The use of plants (leaves, stems, barks, roots, and stems) and plant products (decoctions, extracts, etc.) for treatment of human illnesses has a long history dating back to (around 5,000 years) 3,000 BC (Raskin et al., 2002). According to the World Health Organization (WHO), an impressive 80% of the global population in developing countries depends on complementary and alternative medicine or a traditional system of medicine for their wellbeing and good health (Farnsworth et al., 1985; WHO, 2019). Plants were the main source of remedies for the world during the 1770s as there were only few or no chemically synthesized drugs available (Duke, 1990). Out of 1,031 novel chemical compounds with potential therapeutic values discovered between 1980 and 2002, a total of 600–650 compounds (around 60%) were identified and characterized from natural origin (Newman et al., 2003). Currently, more than 100 compounds isolated and characterized from higher plants having proven pharmacological activities including quinine, atropine, morphine, digitalis, etc. are used in allopathic systems of medicine (Cox, 1994).

In recent years, more and more people from both developing and developed nations are moving towards herbal medicines as their choice for healthcare since they are...
less expensive and have fewer side effects. Hence, there is a resurgence in the scientific research related to use of herbal extracts, isolation, and characterization of active principle(s) and understanding their mechanism of action in ameliorating diseases (Hamdan and Afifi, 2004). Also, the study of plant extracts as protective (antimutagenic, antigenotoxic, and anticlastogenic) agents against environmental carcinogens, mutagens, and chemotherapeutic drugs has gained interest due to the presence of varied phyto-constituents such as flavonoids, alkaloids, coumarins, phenolics, etc. with a wide range of pharmacological properties.

*Andrographis paniculata* (family: Acanthaceae) is an important medicinal herb—used in Indian system of medicine (Ayurveda, Siddha, and Unani), traditional and folk system of medicine. The plant has been used to treat snakebite, malaria, constipation, and also as an appetizer. It has been used in the Asian continent to treat various infections and it is the sole remedy to treat malaria until the discovery of 'quine' drug from *Cinchona*. This plant is included in Indian Pharmacopoeia and it is one of the active ingredients of about 26 Ayurvedic formulations (Sanjutha et al., 2008; Verma et al., 2018). The whole plant, leaves, powdered plant sample, and extract isolated from *A. paniculata* is shown in Fig. 1.

Currently, more than 100 chemotherapeutic drugs are known and are used either as a single entity or in combination of two or more to treat cancer patients. Cyclophosphamide (CPA) is an alkylating agent that belongs to the class of oxazaphosphorines. WHO listed CPA as one of the most successful chemotherapeutic drugs on WHO Model List of Essential Medicines (WHO, 2015). Despite its immunosuppressant and genotoxic properties, CPA has been extensively used to treat a variety of malignancies such as carcinoma of the breasts, lungs, lymphomas, leukemias, and myeloma (Tripathi and Jena, 2008). Two active metabolites of CPA, namely acrolein and phosphoramidemustard formed by the action of liver microsomal enzyme cytochrome P<sub>450</sub> are responsible for the anticancer potential and associated genotoxic effects of this drug (Colvin and Hait, 2010; Kour et al., 2017). The Organization for Economic Co-operation and Development (OECD, 2014) has recommended the use of CPA as a positive control for *in vitro* evaluation of mammalian chromosomal aberrations (CAs) and in genetic toxicity studies.

Based on the pharmacological properties of *A. paniculata* collected through our literature survey, particularly the antioxidant and free radical scavenging properties desirable for antimutagenic activity, this study was performed on the antimutagenic and antigenotoxic consequences of this plant extract against CPA induced genotoxicity on human peripheral lymphocytes *in vitro*. Qualitative phytochemical screening, quantitative estimation of total phenolic contents (TPC), and gas chromatography-mass spectrometry (GC-MS) analysis of the extract were also observed to shed light on its phytoconstituents and bioactive principles.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Roswell Park Memorial Institute (RPMI)-1640, fetal calf serum, and phytohaemagglutinin-P were procured from Gibco BRL (Invitrogen Co., Carlsbad, CA, USA). Colchicine (Loba Chemie Pvt Ltd., Mumbai, India), Giemsa stain solution [E. Merck (India) Ltd., Mumbai, India], and CPA [Biochem (India) Ltd., Mumbai, India] were also acquired. 5-Bromo-2-deoxyuridine was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals and solvents used were of analytical grade and highest purity available.

**Collection of plant materials**

*A. paniculata* was collected from Medavakkam, Chennai,
Kancheepuram, Tamil Nadu, India during the month of October 2018 and the plant authentication was done at Siddha Central Research Institute, Central Council for Research in Siddha, Ministry of Health and Family Welfare, Government of India, Arumbakkam, Chennai, India.

Preparation of EAP
The extract was prepared using methods described by Stefanović et al. (2015) with slight modifications. The powdered plant material (leaves) was extracted by maceration with ethanol. Plant material (50 g) was soaked with 500 mL of ethanol for 24 h at room temperature and then filtered. The residue from the filtration was extracted again, twice, using the same procedure. The filtrates obtained were combined and then evaporated to dryness using a rotary evaporator at 40°C. The extract was stored in sterile tubes at 20°C until needed.

Qualitative phytochemical screening
Preliminary phytochemical analysis was performed on the ethanol extract for the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins, phenolic compounds, and terpenoids by different methods (Khandelwal and Sethi, 2014).

Determination of TPC
The amount of total phenolic content of EAP was determined according to the method of Singleton et al. (1999) with slight modifications. One hundred microliter of extract (concentration 20 μg/mL) was mixed with 0.2 mL of Folin-Ciocalteu reagent, 2 mL of distilled water, and 1 mL of 15% sodium carbonate solution. After 2 h of incubation at room temperature, optical density (OD) values were measured at 765 nm using UV-visible spectrometer. A calibration graph was plotted using different concentrations of gallic acid (on x axis) as standard and their corresponding OD values (on y axis). The total phenolics were extrapolated from the graph and expressed as mg of gallic acid equivalent (mg GAE) per g of extract, dry weight.

GC-MS analysis
Powdered plant material (5 g) was soaked in 95% ethanol overnight and then filtered through a Whatman no. 41 filter paper. Two grams of sodium sulphate was added to the filtrate to remove traces of water and the filtrate is then concentrated by bubbling nitrogen gas into the solution. GC-MS analysis of the extract was performed using a Perkin Elmer GC Clarus 500 system and Gas Chromatograph (PerkinElmer, Inc., Waltham, MA, USA) interfaced to a mass spectrometer equipped with an Elite-5MS fused silica capillary column (30×0.25 mm I.D. ×1 mm dₘ, composed of 5% diphenyl/95% dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier at a constant flow rate of 1 mL/min and an injection volume of 3 μL was employed (split ratio of 10:1). Injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 200°C, then 5°C/min to 280°C ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV, a scan interval of 0.5 s, and fragments from 45 to 450 Da. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass version 5.2.0 (PerkinElmer, Inc.). Interpretation on mass-spectrum GC-MS was conducted using the database of National institute Standard and Technology (NIST) having over 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library and the molecular weight and structure of the components of the test materials were ascertained.

Set up of lymphocyte culture
Heparinised peripheral blood samples were obtained from healthy donors, who were non-smoking, nonalcoholic and not under any medications; not exposed to any mutagens. Informed consent was obtained from each donor. For lymphocyte culture, 0.5 mL blood sample was added to sterile culture vial containing 5 mL of RPMI-1640 medium supplemented with 1.0 mL of fetal calf serum (15%) and 0.1 mL of phytohemagglutinin-P. The culture vials were tightly corked and incubated at 37°C for 24 h.

Analysis CAs
After 24 h of incubation, the lymphocytes were treated individually with different concentrations of EAP (50, 100, 150, 200, and 250 mg/mL), CPA (100 μg/mL), and also co-treated with CPA+EAP (50, 100, 150, 200, and 250 mg/mL). The normal controls (untreated cultures) were also run simultaneously. The culture vials were incubated for 48 h. One hour before (after 47 h), 0.2 mL of colchicine (0.01%) was added to each vial and incubated at 37°C for 45 min. After incubation, the cultures were centrifuged for 10 min at 1,200 rpm and the supernatant was removed. The cell pellet was added with 5 mL of prewarmed (37°C) potassium chloride hypotonic solution (0.075 M). Cells were resuspended and incubated at 37°C for 20 min. After hypotonic treatment, the cultures were centrifuged and the supernatant was discarded. The lymphocytes were fixed by adding ice cold fixative solution comprised of methanol and acetic acid in the ratio of 3:1. The slides were prepared using standard method described by Rothfels and Siminovitch (1958) and stained with Giemsa staining solution as per the protocol de-
scribed by Moorhead et al. (1960). During observations and for calculating CAs, the gaps were not included according to Mace et al. (1978). The number of CAs was obtained by calculating the percentage of metaphases from control, individually treated and co-treated, and for each concentration tested that showed structural alterations. The CA was classified according to the International System for Human Cytogenetic Nomenclature (Paz-y-Miño et al., 2002). CAs were evaluated in 200 well spread metaphases per concentration (a total 100 metaphases per donor). The results were expressed as the number of CAs/cell and % of abnormal cells.

**Analysis of sister chromatid exchanges (SCE)**

For SCE analysis, the lymphocyte culture was set up as follows: Briefly, 0.5 mL blood sample was added to a sterile culture vial containing 5 mL RPMI-1640 medium supplemented 5-bromo-2-deoxyuridine (3 μg/mL), tightly corked and incubated at 37°C for 24 h. After 24 h of incubation, the lymphocytes were treated individually with different concentrations of EAP (50, 100, 150, 200, and 250 mg/mL), CPA (100 μg/mL) alone, and co-treated with CPA+EAP (50, 100, 150, 200, and 250 mg/mL). The normal controls (untreated cultures) were also run simultaneously. The culture vials were incubated for 48 h. The cultures were exposed to colchicine (0.06 μg/mL) for 1 h before harvesting. Hypotonic treatment with potassium chloride and fixation were done in the same way as described earlier for CA. The slides for microscopic observation were processed according to the method described earlier by Perry and Wolff (1974). For each concentration of EAP treated, CPA+EAP co-treated, normal control and CPA, 50 metaphases were scored for SCE. The results were expressed as mean SCE per cell.

**Data analysis**

The data were analyzed using Student’s t-test for statistical significance of all the parameters after one-way analysis of variance (ANOVA) test using SPSS version 16 (SPSS Inc., Chicago, IL, USA). Dose response relationships were determined from the correlation (r) and regression coefficients for the percentage of abnormal cells and SCEs.

**RESULTS**

**Phytochemical screening**

Phytochemical screening of *A. paniculata* extract showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins, phenolic compounds, and terpenoids (Table 1).

| No. | Phytochemical constituent | Test                      | Inference |
|-----|---------------------------|---------------------------|-----------|
| 1   | Alkaloids                 | Wagner’s test             | ++        |
|     |                            | Mayer’s test              |           |
| 2   | Carbohydrates             | Molisch’s test            | +         |
|     |                            | Fehling’s test            |           |
| 3   | Flavonoids                | Shinoda test              | ++        |
| 4   | Glycosides                | Borntrager’s test         | ++        |
|     |                            | Legal’s test              |           |
|     |                            | Keller-Killiani test      |           |
| 5   | Saponins                  | Froth test                | +         |
| 6   | Steroids                  | Salkowski reaction        | +++       |
| 7   | Tannins and phenolic compounds | Ferric chloride test | ++       |
|     |                            | Lead acetate test         |           |
|     |                            | Nitric acid test          |           |
| 8   | Terpenoids                | Libermann-Burchard’s test | +         |

+, mildly present; ++, highly present; ++++, more highly present.

**TPC**

Plant phenolics are known for health promoting potentials due to their excellent antioxidant and free radicals scavenging activities. In the present study, the phenolic content of *A. paniculata* extract was quantified and found to be 35.66±3.42 mg GAE/g of extract and the yield of extract was found to be 15.24±1.76% (Table 2).

**GC-MS analysis**

The ethanol extract of *A. paniculata* contained rich phytochemical constituents evidenced from phytochemical screening. GC-MS analysis of the extract showed a total of 11 different major compounds. The prevailing phytoconstituents along with their retention time (RT) are presented in Table 3 and Fig. 2. The major bioactive compounds identified in the extract are n-hexadecanoic acid, tert-decanoic acid, phytol, oleic acid, octadecanoic acid, stigmasterols, and sitosterols.

**Structural chromosomal aberrations (SCAs)**

The beneficial effects of EAP on reducing CAs caused by CPA are presented in Table 4. The lymphocyte cultures which had 48 h continuous exposures to *A. paniculata*
Table 3. Major phytochemical components identified by gas chromatography mass spectrometry (GC-MS) analysis from ethanol extract of *Andrographis paniculata*.

| Retention time | Peak area (%) | Name of the compound | Library ID |
|----------------|---------------|----------------------|------------|
| 16.448         | 3.89          | Bicyclo[3.1.1]heptanes, 2,6,6-trimethyl-(1-α,2-β,5-α) | 17013 006876 13 760 |
|                |               | Bicyclo[3.1.1]heptane, 2,6,6-trimethyl- | 16968 000473 55 255 |
|                |               | Bicyclo[3.1.1]heptane, 2,6,6-trimethyl- [1R-(1-α,2-β,5-α)] | 17016 004795 86 250 |
| 17.320         | 1.45          | Hexadecanoic acid, methyl ester | 119400 10 39 98 |
|                |               | Hexadecanoic acid, methyl ester | 000112 39 98 |
|                |               | Hexadecanoic acid, methyl ester | 119406 39 97 |
|                |               | Hexadecanoic acid, methyl ester | 119408 39 97 |
| 17.683         | 19.79         | n-Hexadecanoic acid | 107549 10 39 98 |
|                |               | n-Hexadecanoic acid | 000057 10 39 98 |
|                |               | n-Hexadecanoic acid | 119408 10 39 98 |
| 19.135         | 7.45          | Phytol | 141393 86 79 99 |
|                |               | Phytol | 000150 86 79 99 |
|                |               | Phytol | 141395 86 77 4 |
| 19.353         | 15.06         | Oleic acid | 129338 000112 80 199 |
|                |               | 9-Octadecenoic acid (E) | 129353 000112 79 899 |
|                |               | 6-Octadecanoic acid | 129340 66 89 8 |
| 19.556         | 4.04          | Octadecanoic acid | 131262 000057 11 498 |
|                |               | Octadecanoic acid | 131261 000057 11 496 |
|                |               | Octadecanoic acid | 131258 000057 11 496 |
| 23.201         | 13.91         | 2-Amino-4-morpholino-6-phenylcarbamoyl-1,3,5-triazine | 144180 007537 63 553 |
|                |               | Ethanone, 1-(4-hydroxy-3,5-dimethoxy phenyl)- | 57688 002478 38 845 |
|                |               | Ethanone, 1-(4-hydroxy-3,5-dimethoxy phenyl)- | 57687 002478 38 843 |
| 24.015         | 2.36          | Anthranilic acid, N-methyl-, butyl ester | 66844 015236 34 743 |
|                |               | 1,2-Benzisothiazol-3-amine tert-butylimethylsilyl | 113699 57 238 |
|                |               | Hexahydropyridine, 1-methyl-4(4,5) | 1000332 47 138 |
| 26.208         | 4.73          | 1,2-Benzothiazol-3-amine tert-butylimethylsilyl | 113699 57 250 |
|                |               | 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene | 129190 093103 70 946 |
|                |               | 5-Methyl-2-phenylindololozine | 129190 093103 70 946 |
| 28.212         | 8.18          | Stigmasterol | 216703 000083 48 756 |
|                |               | tert-Butyl(5-isopropyl-2-methylphenoxy) dimethylsilane | 114128 02 338 |
|                |               | Cyclohexane carboxamid, N-furfuryl | 1000367 32 838 |
| 28.793         | 28.61         | γ-Sitosterol | 217434 000083 47 690 |
|                |               | β-Sitosterol | 217432 000083 46 556 |
|                |               | Stigmast-7-en-3-ol, (3-β,5-α,24S) | 217448 018525 35 443 |

The extract (50∼250 mg/mL) did not show any changes in CAs, when compared to normal control. This indicated the non-mutagenic nature of the plant extract. The CPA alone at a concentration of 100 µg/mL significantly increased the number of abnormal metaphases and the total number of structural CAs in lymphocytes, when compared to normal control (*P*<0.01). In contrast, the cells co-treated with CPA+EAP showed a significant reduction (*P*<0.05 or *P*<0.01) in SCAs per cell and the % of abnormal cells, when compared to CPA alone treated cells. This indicates the antigenotoxic potential of the extract against CPA.

**SCE**

Similar results (as that of CAs) were obtained for SCE and the results are presented in Table 5. The lymphocyte cultures exposed to *A. paniculata* extract (50∼250 mg/mL) did not show any changes in SCE, when compared to
Antigenotoxic Potential of *Andrographis paniculata*

**Table 4.** The structural chromosomal aberrations (CAs) and percentage of abnormal cells in human lymphocytes treated with cyclophosphamide (CPA) alone, *Andrographis paniculata* (EAP) alone and CPA+EAP after 48 h treatment

| Treatment          | Chromatid breakage | Chromosome breakage | Structural CA/cell | % of abnormal cells |
|--------------------|--------------------|---------------------|--------------------|---------------------|
| Normal control     | 11                 | 3                   | 0.07               | 6.50±0.50           |
| CPA (100 µg/mL)    | 38                 | 16                  | 0.27**             | 26.50±2.50**        |
| EAP 50 mg/mL       | 9                  | 4                   | 0.07               | 6.50±1.50           |
| EAP 100 mg/mL      | 10                 | 2                   | 0.06               | 5.50±0.50           |
| EAP 150 mg/mL      | 12                 | 5                   | 0.08               | 8.00±1.00           |
| EAP 200 mg/mL      | 12                 | 6                   | 0.09               | 8.50±1.50           |
| EAP 250 mg/mL      | 14                 | 5                   | 0.10               | 9.00±1.50           |
| CPA+EAP 50 mg/mL   | 33                 | 12                  | 0.23***            | 21.50±1.00**        |
| CPA+EAP 100 mg/mL  | 29                 | 12                  | 0.21***            | 20.50±1.50**        |
| CPA+EAP 150 mg/mL  | 24                 | 9                   | 0.17***            | 15.50±1.50***       |
| CPA+EAP 200 mg/mL  | 20                 | 9                   | 0.15***            | 13.50±1.50***       |
| CPA+EAP 250 mg/mL  | 17                 | 7                   | 0.12***            | 11.00±1.00***       |

Significantly different from normal control at *P<0.05 and **P<0.01, and positive control (CPA) at *P<0.05 and ##P<0.01. CAs were evaluated in 200 well spread metaphases per concentration. A total 100 well spread metaphases per donor, no. of donors = 2.

**Table 5.** The SCEs per chromosome and sister chromatid exchanges (SCEs) per cell in human lymphocytes treated with cyclophosphamide (CPA) alone, *Andrographis paniculata* (EAP) alone and CPA+EAP after 48 h treatment

| Treatment          | No. of metaphases scored | Total no. of SCEs scored | Mean SCEs / chromosome | SCEs/cell (mean±SE) |
|--------------------|--------------------------|--------------------------|------------------------|---------------------|
| Normal control     | 50                       | 127                      | 0.0552                 | 2.54±0.12           |
| CPA (100 µg/mL)    | 50                       | 496                      | 0.2156**               | 9.92±0.63**         |
| EAP 50 mg/mL       | 50                       | 120                      | 0.0521                 | 2.40±0.08           |
| EAP 100 mg/mL      | 50                       | 124                      | 0.0539                 | 2.48±0.06           |
| EAP 150 mg/mL      | 50                       | 119                      | 0.0508                 | 2.34±0.06           |
| EAP 200 mg/mL      | 50                       | 126                      | 0.0547                 | 2.52±0.11           |
| EAP 250 mg/mL      | 50                       | 131                      | 0.0569                 | 2.62±0.05           |
| CPA+EAP 50 mg/mL   | 50                       | 419                      | 0.1821***              | 8.38±0.14**         |
| CPA+EAP 100 mg/mL  | 50                       | 376                      | 0.1634***              | 7.52±0.11**         |
| CPA+EAP 150 mg/mL  | 50                       | 310                      | 0.1347***              | 6.20±0.13**         |
| CPA+EAP 200 mg/mL  | 50                       | 274                      | 0.1191***              | 5.48±0.20**         |
| CPA+EAP 250 mg/mL  | 50                       | 228                      | 0.0991***              | 4.56±0.18**         |

Significantly different from normal control at *P<0.05 and **P<0.01, and positive control (CPA) at *P<0.05 and ##P<0.01. A total 50 well spread metaphases/cultures were scored for the occurrence of SCE, no. of donors = 2.

**DISCUSSION**

Due to modern lifestyle, industrialization and global environmental pollution, there has been an increase in the incidence rate of chromosomal/gene mutations, which leads to cancers of different organs. The ways and means to counteract the effects of such environmental pollutants and mutagenic agents is to discover the natural com-

normal control. However, the cells co-treated with CPA +EAP showed a significant reduction (*P<0.05 or *P<0.01) in SCEs per chromosome and mean SCEs/cell compared to CPA treated cells. The results again reaffirm the antimutagenic nature and antigenotoxic potential of the extract against CPA.
pounds that can neutralize their mutagenic properties. In this context, medicinal herbs are the promising sources of antimutagenic agents, as they possess wide varieties of phytochemicals in the form of secondary metabolites (Ammar et al., 2007). A. paniculata is a traditional medicinal plant; particularly its aerial parts and leaves are extensively used in Ayurvedic medicines.

The phytochemical screening methods are of supreme importance in identifying new sources of pharmacologically important compounds having therapeutic values and to make the best and sensible use of available natural resources (Mungole et al., 2010). This study examined the phytochemical composition of ethanol extract from A. paniculata. The results revealed that the extract is rich in tannins, saponins, flavonoids, terpenoids, alkaloids, and steroids. The phenolic compounds are one of the largest and most ubiquitous plant metabolites reported to have potential health benefits due to their antioxidant nature. The total phenolic content of A. paniculata was found to be 35.66±3.42 mg of gallic acid equivalent/g of extract. The high amount of total phenolic content observed in the extract justifies its health promoting effects owing to its antioxidant activities. A total of 11 major bioactive compounds including n-hexadecanoic acid, tert-decanoic acid, phytol, oleic acid, octadecanoic acid, stigmasterols, and sitosterols were identified in the extract using GC-MS analysis.

The cytogenetic studies involve assessment of CA(s) at metaphase stage, which gives a more accurate picture of the antimutagenic and anticlastogenic activity of the plant extracts or isolated compounds (Siddique and Afzal, 2005). It has been reported that human lymphocytes are exceptionally sensitive indicators of the in vitro assay systems. In the present study, lymphocyte culture was used to evaluate the antimutagenic and antigenotoxic effects of EAP and the parameters analyzed were CAs and sister chromatid exchanges.

The structural CAs and SCEs were analyzed both in the presence as well as in the absence of ethanol extract from A. paniculata. This plant was chosen because it contained a range of terpenoids (e.g. andrographolide), which are reported to induce differentiation of cancer cells. Furthermore, the plant extract has been reported to exert a broad-range of antiproliferative activity on a variety of cancer cell lines including Michigan Cancer Foundation-7 breast cancer cell lines, colon, cervical, prostate cancers, and leukemia (Geethangili et al., 2008). The lymphocyte cultures treated with different concentrations of EAP (50 ~ 250 mg/mL) did not show any significant changes in CAs and SCEs. In addition, the genotoxic effects of CPA were significantly reduced in lymphocytes co-treated with CPA+EAP. These results substantiate that A. paniculata is non mutagenic and has a protective effect against CPA induced genotoxicity.

From the above results, it can be inferred that EAP was non mutagenic and not genotoxic at all five concentrations tested. Furthermore, EAP exerted an excellent beneficial effect against genotoxic impacts of the known mutagen and chemotherapeutic agent CPA. Our results corroborate previous studies on antigenotoxic effect of A. paniculata, in which the plant has been reported to ameliorate the aflatoxin B1-induced toxicity in vivo and in vitro experiments (Ahmad et al., 2014). Another in vivo study has reported that the 70% ethanol extract of A. paniculata increased the life spans of mice injected with thymoma cells (Zhou et al., 2006). Earlier studies on the anticancer mechanism of several compounds reported that they might also act through an antimutagenic mechanism. Therefore, the search for antimutagenic compounds from natural sources and divulging their mechanism of action represents a fast-growing field of cancer research (Ikken et al., 1999). The herbal extracts and natural compounds having antimutagenic properties may be supplemented with chemotherapy to antagonize the toxic side effects associated with the chemotherapeutic drugs (Heo et al., 2001; Ferguson and Philpott, 2008; El-Sayed et al., 2013).

The antimutagenic activity of EAP observed in the present study might be due to three different mechanisms either within or outside the cells and explained as follows: i) through interrupting the mutagenic agent before it reaches the target cell, ii) through preventing the formation of an active metabolite, and iii) by modulating the mutagenic events after the initiation of DNA damage.

In summary, the findings from this study show that A. paniculata extract is rich in flavonoids, phenolic contents, and other bioactive constituents. The results of the study clearly indicated that A. paniculata is antimutagenic in nature and had a protective effect against CPA induced genotoxicity. From the results, it is reasonable to conclude that the consumption of A. paniculata is safe and can protect cells from a variety of environmental mutagens and carcinogens. However, further studies on isolation and characterization of specific bioactive principle(s) and their mechanism of action in other in vivo models are warranted and under progress in our laboratory. A. paniculata can be used in reducing the risks of cancers and also as a supplement with chemotherapeutic drugs to reduce their toxic side effects.

ACKNOWLEDGEMENTS

The authors are sincerely thankful to Kavin Bioresearch, Chennai, India for providing GC-MS analyses of the extract and to Central Council for Research in Siddha, Department of AYUSH, Ministry of Health and Family Welfare, Government of India for plant authentication.
AUTHORS’ CONTRIBUTIONS

PS and PM performed the experiments, analyzed/interpreted data and wrote the manuscript. RS and NS assisted in performing experiments and writing the manuscript. AP contributed to the concept, designed experiments, analyzed/interpreted data, and finalized the manuscript.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

Ahmad MS, Ahmad S, Arshad M, Afzal M. Andrographis paniculata a miracle herbs for cancer treatment: in vivo and in vitro studies against aflatoxin B1 toxicity. Egypt J Med Hum Genet. 2014. 15:163-171.

Ammar RB, Bouhlel I, Valenti K, Sghaier MB, Kilani S, Mariotte AM, et al. Transcriptional response of genes involved in cell defense system in human cells stressed by H2O2 and pre-treated with (Tunisian) Rhamnus alaternus extracts: combination with polyphenolic compounds and classic in vitro assays. Chem Biol Interact. 2007. 168:171-183.

Colvin M, Hait WN. Alkylating agents and platinum antitumor compounds. 8th ed. In: Hong WK, Bast RC Jr, Hait WN, Kufe DW, Pollock RE, Weichselbaum RR, et al., editors. Holland-Frei Cancer Medicine. People’s Medical Publishing House-USA, Shelton, CT, USA. 2010. p 633-644.

Cox PA. The ethnobotanical approach to drug discovery: strengths and limitations. Ciba Found Symp. 1994. 185:25-41.

Duke JA. Promising phytomedicinals. In: Janick J, Simon JE, editors. Advances in New Crops. Timber Press, Portland, OR, USA. 1990. p 491-498.

El-Sayed WM, Hussain WA, Al-Faiyz YS, Ismail MA. The position of imidazopyridine and metabolic activation are pivotal factors in the antimutagenic activity of novel imidazo[1,2-a]pyridine derivatives. Eur J Pharmacol. 2013. 715:212-218.

Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal plants in therapy. Bull World Health Organ. 1985. 63:965-981.

Ferguson LR, Philpott M. Nutrition and mutagenesis. Annu Rev Nutr. 2008. 28:313-329.

Geethangili M, Yao RK, Fang SH, Zheng YM. Cytotoxic constituents from Andrographis paniculata induce cell cycle arrest in Jurkat cells. Phytother Res. 2008. 22:1336-1341.

Hamdan II, Afifi FU. Studies on the in vitro and in vivo hypoglycemic activities of some medicinal plants used in treatment of diabetes in Jordanian traditional medicine. J Ethnopharmacol. 2004. 93:117-121.

Heo MY, Sohn SJ, Au WW. Anti-genotoxicity of galangin as a cancer chemopreventive agent candidate. Mutat Res. 2001. 488:135-150.

Ikken Y, Morales P, Martínez A, Marín ML, Haza AI, Cambero MI. Antimutagenic effect of fruit and vegetable ethanolic extracts against N-nitrosamines evaluated by the Ames test. J Agric Food Chem. 1997. 45:3257-3264.

Khandelwal KR, Sethi VK. Practical pharmacognosy: techniques and experiments. 24th ed. Nirali Prakashan, Pune, India. 2014. p 149-156.

Kour J, Ali MN, Ganaie HA, Tabassum N. Amelioration of the cyclophosphamide induced genotoxic damage in mice by the ethanolic extract of Equisetum arvense. Toxicol Rep. 2017. 4:226-233.

Mace ML Jr, Daskal Y, Wray W. Scanning-electron microscopy of chromosome aberrations. Mutat Res. 1978. 52:199-206.

Moorehead PS, Nowell PC, Melman WJ, Battrips DM, Hungerford DA. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res. 1960. 20:613-616.

Mungole AJ, Awati R, Chaturvedi A, Zanwar P. Preliminary phytochemical screening of Ipomoea obscura (L) – a hepatoprotective medicinal plant. Int J PharmTech Res. 2010. 2:2307-2312.

Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. J Nat Prod. 2003. 66:1022-1037.

OECD. 2014. OECD guideline for the testing of chemicals: in vitro mammalian chromosomal aberration test. TG 473. Organisation for Economic Co-operation and Development, Paris, France. [cited 2019 Jun 04] Available from: https://ntp.niehs.nih.gov/iccvam/suppdocs/tedocs/oecd/oecd-tg473-2014-058.pdf

Paz-y-Miño C, Bustamante G, Sánchez ME, Leone PE. Cytogenetic monitoring in a population occupationally exposed to pesticides in Ecuador. Environ Health Perspect. 2002. 110:1077-1080.

Perry P, Wolf S. New Giemsa method for the differential staining of sister chromatids. Nature. 1974. 251:156-158.

Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulve A, Borisjuk N, et al. Plants and human health in the twenty-first century. Trends Biotechnol. 2002. 20:522-531.

Rothfels KH, Siminovitch L. A dry-air driving technique for flattening chromosomes in mammalian cells grown in vitro. Stain Technol. 1958. 33:73-77.

Sanjutha S, Subramaniam S, Indu Rani C, Maheswari J. Integrated nutrient management in Andrographis paniculata. Res J Agric Biol Sci. 2008. 4:141-145.

Siddiqui YH, Afzal M. Protective role of allicin and L-ascorbic acid against the genotoxic damage induced by chloramidine acetate in cultured human lymphocytes. Indian J Exp Biol. 2005. 43:769-772.

Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method Enzymol. 1999. 299: 152-178.

Stefanović OD, Tešić JD, Čomić LR, Miltiades albus and Dorycnium herbaeum extracts as source of phenolic compounds and their antimicrobial, antibiofilm, and antioxidant potentials. J Food Drug Anal. 2015. 23:417-424.

Tripathi DN, Jena GB. Astaxanthin inhibits cytotoxic and genotoxic effects of cyclophosphamide in mouse germ cells. Toxicol. 2008. 248:96-103.

Verma H, Negi MS, Mahapatra BS, Shukla A, Paul J. Evaluation of a bioactive principle from Aegle marmelos for neurodegenerative disease. J Ethnopharmacol. 2013. 149-156.

WHO. WHO global report on traditional and complementary medicine 2019. World Health Organization, Geneva, Switzerland. 2019. p 45-80.

WHO. WHO model list of essential medicines. 19th List. [cited 2020 Mar 12]. Available from: https://www.who.int/selection_medicines/committees/expert/20/EML_2015_HI_NAL_amended_AUG2015.pdf?ua=1

Zhou J, Zhang S, Ong CN, Shen HM. Critical role of pro-apoptotic Bcl-2 family members in andrographolide-induced apoptosis in human cancer cells. Biochem Pharmacol. 2006. 72:132-144.