Prev. Nutr. Food Sci. 2020;25(3):246-253
https://doi.org/10.3746/pnf.2020.25.3.246
pISSN 2287-1098 · eISSN 2287-8602

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

Ayyakkannu Purushothaman[1], Parveen Sufiya[1], Packirisamy Meenatchi[1], Ramalingam Sundaram[1], and Nallappan Saravanan[2]

[1]Post-Graduate and Research Department of Biochemistry, Mohamed Sathak College of Arts and Science, University of Madras, Chennai, Tamil Nadu 600119, India
[2]Department 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 of extract (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) 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 heath care since they are

Received 19 May 2020; Accepted 15 June 2020; Published online 30 September 2020

Correspondence to Ayyakkannu Purushothaman, Tel: +91-44-2450-1115, E-mail: purushothamanbiochem@gmail.com
Author information: Ayyakkannu Purushothaman (Professor), Parveen Sufiya (Researcher), Packirisamy Meenatchi (Professor), Ramalingam Sundaram (Researcher), Nallappan Saravanan (Professor)

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 'quinine' 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 phosphoramide mustard formed by the action of liver microsomal enzyme cytochrome P_{450} 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,

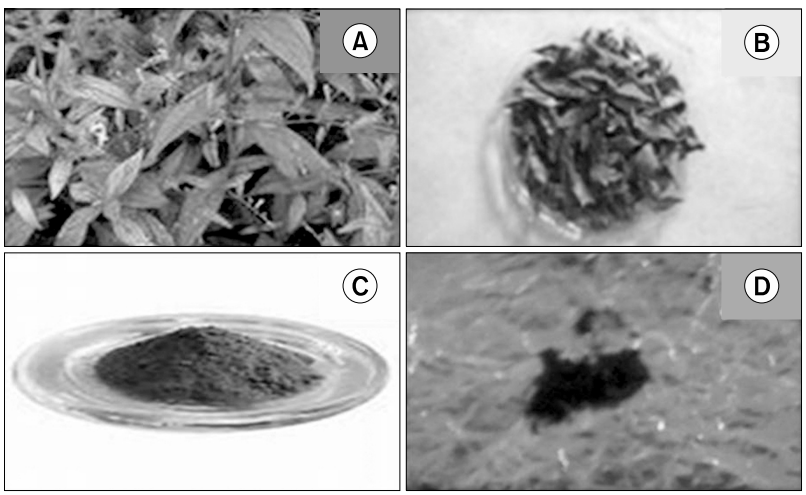

Fig. 1. Photographs showing *Andrographis paniculata*. (A) Whole plant, (B) shade dried leaves, (C) powdered sample, and (D) ethanol extract.

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 d_f, 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).

Table 1. The phytochemical components in ethanol extract of *Andrographis paniculata* based on preliminary phytochemical screening tests

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 2. Extract yield and total phenolics content for *Andrographis panicuata*

Sample	Extract yield (%)	Total phenolics (mg GAE/g)
Extract of *A. paniculata*	15.24±1.76	35.66±3.42

Each value is expressed as mean±SD from minimum of three independent experiments.
GAE, gallic acid equivalent.

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	39	98
		Hexadecanoic acid, methyl ester	000112	39	98
		Hexadecanoic acid, methyl ester	119406	39	97
			000112		
			119408		
			000112		
17.683	19.79	*n*-Hexadecanoic acid	107549	10	399
		n-Hexadecanoic acid	000057	10	398
		tert-Decanoic acid	107548	63	893
			000057		
			84453 000544		
19.135	7.45	Phytol	141393	86	799
		Phytol	000150	86	790
		Phytol	141393	86	774
			000150		
			141395		
			000150		
19.353	15.06	Oleic acid	129338 000112	80	199
		9-Octadecenoic acid (E)	129353 000112	79	899
		6-Octadecenoic acid	129340	66	898
			1000336		
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*-butyldimethylsilyl	113699	57	238
		Hexahydropyridine, 1-methyl-4-(4,5)	1000332	47	138
			66899 094427		
26.208	4.73	1,2-Benzithiazol-3-amine *tert*-butyldimethylsilyl	113699	57	250
		2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene	1000332	70	946
		5-Methyl-2-phenylindolozine	129190 093103	99	743
			67006 036944		
28.212	8.18	Stigmasterol	216703 000083	48	756
		tert-Butyl(5-isopropyl-2-methylphenoxy) dimethylsilane	114128	02	338
		Cyclohexane carboxamide, N-furfuryl	1000367	32	838
			66823 006341		
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

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

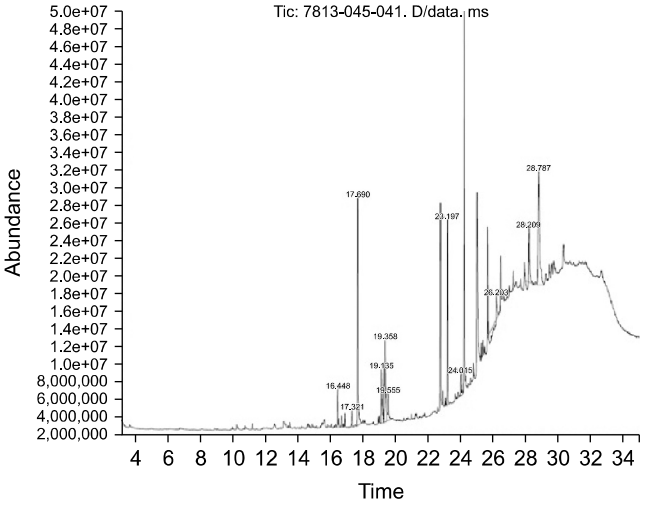

Fig. 2. A representative gas chromatography-mass spectrometry chromatogram of ethanol extract from *Andrographis paniculata*.

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.

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-

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	CA		Structural CA/cell	% of abnormal cells
	Chromatid breakage	Chromosome breakage		
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, ethanol extract of *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.

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 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 B_1-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, Afzald M. *Andrographia 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 H_2O_2 and pretreated 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 conpounds. 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, Hussin 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, Rao YK, Fang SH, Tzeng 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. 1999. 47:3257-3264.

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.

Moorhead PS, Nowell PC, Mellman WJ, Battips 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/feddocs/oecd/oecd-tg473-2014-508.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, Wolff S. New Giemsa method for the differential staining of sister chromatids. Nature. 1974. 251:156-158.

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

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

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

Siddique YH, Afzal M. Protective role of allicin and L-ascorbic acid against the genotoxic damage induced by chlormadinone 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. *Melilotus albus* and *Dorycnium herbaceum* 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 mice germ cells. Toxicology. 2008. 248:96-103.

Verma H, Negi MS, Mahapatra BS, Shukla A, Paul J. Evaluation of an emerging medicinal crop Kalmegh [*Andrographis paniculata* (Burm. F.) Wall. Ex. Nees] for commercial cultivation and pharmaceutical & industrial uses: a review. J Pharmacog Phytochem. 2019. 8:835-848.

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

WHO. 2015. 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_FINAL_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.