Research Article

Callus Cytology and Electrophoretic Protein Profiling in Cultured Cells in *Andrographis paniculata* (L.)

[1]Vijay Damodhar Mendhulkar*, [2]Abhijeet Jayadev Mayekar, [3]Aruna Shahaji Nangare
[1]Professor and Head, Department of Botany, Institute of Science, Mumbai, India.
[2]Department of Botany, Institute of Science, Mumbai, India.
[3]Research Scholar, Department of Botany, Institute of Science, Mumbai, India.
***Corresponding author's E-mail:** drmendhulkar@gmail.com

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ABSTRACT

Andrographis paniculata is a herb widely used in traditional system of medicine, also known as king of bitters. The present work deals with the comparative study of protein profiling in *in-vitro* (callus cells) and *in-vivo* grown cells and mitotic chromosomal status in *Andrographis paniculata* (L.). Callus cytology was studied to observe whether controlled conditions induce changes at biochemical and genetic level. Total protein content was estimated by Lowry's method in the leaf sample and it was 32µg/gm. While the callus cells generated from leaf showed 14µg/gm. of total protein. The leaf sample indicated the presence of total number of nine bands with the RF range of 0.04 to 0.81. The numbers of total bands in the cultured cell sample were eight, with the highest RF value, 0.71 and lowest RF value 0.03. The results obtained on cell cytology of cultured cells indicate the evidence of chromosomal aberrations that mainly includes clumping and grouping of chromosomes, sticky chromosomes, broken bridge, chromosome bridge, chromosome fragments, micronuclei formation, many micronuclei formation. Such anomalies are expected to induce variations at biochemical level and may cause changes in cell metabolism and genetical manifestation therefore, the protein estimation and electrophoretic protein profiling was carried out in studied experimental system *Andrographis paniculata* (L.).

Keywords: *Andrographis paniculata,* SDS-PAGE, RF value, protein profiling.

INTRODUCTION

Andrographis Paniculata is traditionally known as kalmegh. The plant belongs to family Acanthaceae and is widely used in Ayurvedic and Homeopathic systems of medicine. *Andrographis paniculata* is distributed in tropical Asian countries, often in isolated patches. It can be located in a variety of habitats, e.g. plains, hill slopes, waste lands, farms, dry or wet lands, sea shores and even road sides. Unlike other species of the genus, *Andographis paniculata* is of common occurrence in most places in India, including the plains and hilly areas which accounts for its wide use. Since time immemorial, village and ethnic communities in India have been using this herb for treating a variety of ailments. In traditional Chinese medicine this herb used to release body heat in fever.

The diterpene lactone andrographolide was first isolated as a major constituent[1] and later characterized as a lactone.[2] A number of related minor diterpenes and their glycosides have since been identified.[3] When callus cultures of the plant were investigated, andrographolide and the other diterpenes were not produced. Instead, the sesquiterpenes paniculides A-C were found.[4] Other constituents of the plant include various flavones.[5] Extracts of Andrographis and andrographolide derivatives have shown modest activity in vitro against HIV.[5] Inhibition of passive cutaneous anaphylaxis and mast cell stabilization was observed in studies of the purified diterpenes in rats.[6] In clinical studies, the doses of crude plant are administered in the range of 3 to 6 g. Clinical trials in children with upper respiratory tract infection reported the use of andrographolide 30 mg daily for 10 days.[7]

The present studies deals with the study of protein content and protein profiling in leaf samples and callus. Callus cytology was also studied to ascertain chromosomal changes in controlled condition.

MATERIALS AND METHODS

Plant material

The plant was collected from the medicinal plant nursery then grown and habituated in the garden of Institute of Science, Mumbai. The leaf explants of the 7 days old Seedlings of control plant were taken to induce callus in the MS media supplemented with 2-4-D, Kinetin and BAP hormones with different combinations.

Extraction and estimation of proteins

The extractions of proteins were carried out in phosphate buffer.0.1gm of leaf powder and callus was extracted separately in 10ml sterile distilled water boiled for 5min. Cooled and filtered broth was used for protein estimation. Protein content was determined by using method suggested by Lowry.[8] Bovine Serum Albumin (0.1mg/ml) was used as a standard protein. Different volumes with 0.2, 0.4, 0.6, 0.8, 1 ml of standard; 0.2ml of leaf and callus in the test tubes was taken. The volume was adjusted to 1ml with distilled water.5ml of Biuret reagent (50ml of 2% Na_2CO_3 in 0.1N aqueous NaOH+1 ml of 0.5% CuSO4 in 1% potassium sodium tartarate.) was

added to all the test tubes. Addition of 0.5ml diluted Folin's reagent was followed after incubation at room temperature for 10 min. After further incubation at 25°C for 30 min. The blue colour developed was measured by taking OD at 660 nm. A standard graph of BSA was plotted and the amount of protein in the sample was calculated (Graph.1).

Electrophoretic profiling of protein

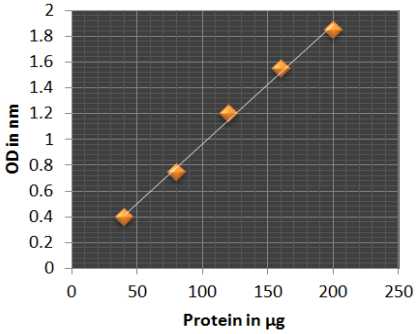
Protein profiling was done by SDS-PAGE method. Samples for electrophoresis were prepared by adding sample buffer (5X) and protein samples in 1:4 ratio. Standard protein marker was also prepared by same method. Samples were heated in boiling water bath for 2 to 3 min. Samples were cooled and carefully injected using micro syringe into wells through electrode buffer. The gel was run in refrigerator for proper cooling of electrode buffer and plates. The gel was run at 50 mA current until the bromophenol blue reaches to the bottom of the gel. After completion of run, the gel was carefully removed from the plates and immersed in staining solution for overnight. The gel was then transferred to destaining solution. After proper destaining the gel was documented by photography and geldoc (Alpha Imager HP, Alphalnotech Corporation).

Callus cytology

The profused callus was observed after 8-10 weeks with the hormonal combination of 2-4-D: BAP in the concentration of 1:0.5 (mg/l).For the callus cytology, the callus tissues of leaf explants were taken just after placing in light conditions for two hours and were fixed in Cornoy's fluid overnight. They were then slightly warmed in a 2% aceto-orcein – (1N) HCL mixture of 9:1 and kept for one hour before squashing in 45% acetic acid. Placed a cover slip and observed under microscope.

RESULTS AND DISCUSSION

The total protein content in leaf was estimated as 32 µg/gm. while it was 14µg/gm in callus cells. The protein content in normal sample was more compared to callus cells. The remarkable variation in the protein content is accountable to degradation of normal protein components, due to the exposure of cells to the controlled condition (under in vitro condition). In contrast Dhabhai and Batra,[9] reported high protein content in callus cells of *Acacia nilotica.*



Graph 1: Standard Graph of Lowry's method.

These variations were also accompanied with the variations in the total band number and RF values in electrophoretic profiling in both the samples (Photoplate-1&2). The normal sample indicates the presence of total number of nine bands with the highest range of RF value 0.81 to the lowest RF value of 0.04. Whereas, the number of total bands in the cultured cell sample were eight, with the highest RF value 0.71 and lowest RF value 0.03. The protein band number 2,3,4,6 & 7 appeared at the same RF value when compared in both the samples. However the protein band number 1, 5 & 8 indicated the variations in the RF value of corresponding bands of studied samples. Protein bands 5 & 7 exhibit RF value 0.40 & 0.59 under in-vivo condition (Table no. 1) which is different from that of *in-vitro* condition with the difference of 0.02. Protein band number 1 under *in-vitro* condition differs with negligible difference in RF value of corresponding band in the sample of *in-vivo* condition.

The variation in band number and RF value is supportive evidence that indicates the effect of cultured conditions at biochemical level.

Mitotic studies

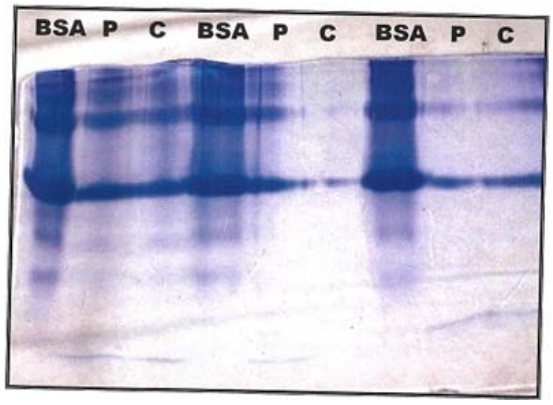
The mitotic cell division studies in the cultured cells of *Andrographis paniculata* showed chromosomal damage and mitotic disturbances, thus providing the evidence of the effect at genetic level. The results obtained indicated structural changes in the chromosomal components. The chromosomal anomalies observed were clumping and grouping of chromosomes, sticky chromosomes, broken bridge, chromosome bridge, chromosome fragments, micronuclei formation, many micronuclei formation (Photo plate:3). The metaphase constituted the major abnormalities. The stickiness of chromosomes at anaphase caused inability of normal movement at anaphase. The fragmentation of chromosome from stress of anaphasic movement. The bridge formation is the failure of chromosome to separate. The maximum frequencies of aberrations were seen in the cells which were grown under *in vitro* condition; compared to *in vivo* condition. Chromosomal aberrations (especially micronucleus, grouping and clumping of chromosomes) were of maximum occurrence. The multipolarity, reorientation, unusually differentiated cytoplasmic matrix and chromatin matrix, were the other anomalies which were observed during the mitotic screening. Few cells with cytomixis also were observed in callus cells.

Chand and Roy [10] have also reported chromosomal aberrations in the callus cells of *Nigella sativa* grown in MS media.

The abnormalities observed indicate clear evidences of possible interaction of media components with the genetic system of cells when grown under controlled condition.

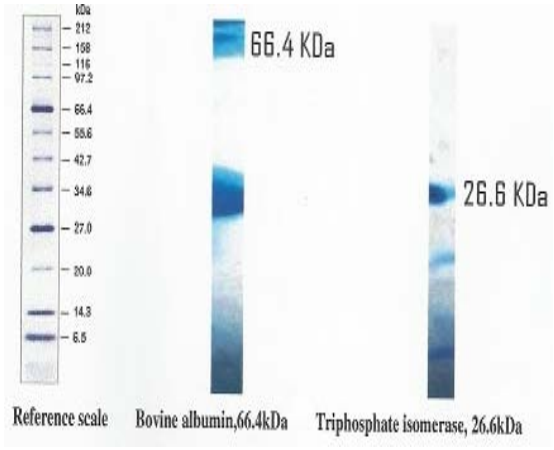
Changes in cell activities are triggered when cells are transferred from one environment to a different environment.[11] The transfer from *in vivo* to *in vitro*

environments may trigger changes in cellular behaviour[12] and it can be best evaluated through cytological studies, such as the determination and comparison of the DNA content, chromosome count, genetic stability, and cell cycle.[13]



Photoplate 1: SDS-PAGE plate for protein bands in studied samples of *Andrographis paniculata*

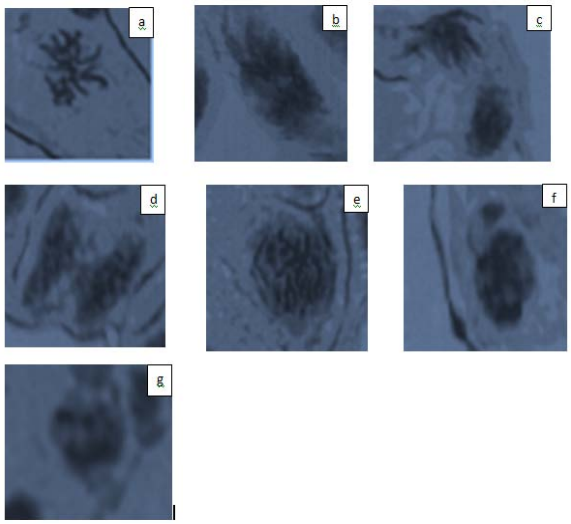
BSA- Bovine Serum Albumin, P- *In vivo* sample of selected plant system, C- *in vitro* sample of selected plant system.



Photoplate 2: Reference scale for standard proteins.

Table 1: Electrophoretic protein binding profile and RF values for Protein bands in studied samples of *Andrographis paniculata*.

Band No.	Distance travelled by each band (mm)		Breadth of the bands (mm)		RF value	
	In Vivo	In Vitro	In Vivo	In Vitro	In Vivo	In Vitro
1	2.0	1.5	1.0	0.5	0.04	0.03
2	4.0	4.0	1.0	1.0	0.08	0.08
3	8.0	8.0	1.5	1.0	0.16	0.16
4	12.0	12.0	2.0	3.0	0.24	0.24
5	20.0	21.0	1.5	1.0	0.40	0.42
6	24.0	24.0	2.0	2.5	0.48	0.48
7	29.0	28.0	2.0	2.5	0.59	0.57
8	35.0	35.0	2.0	2.0	0.71	0.71
9	40.0	Nil	1.0	Nil	0.81	Nil



Photoplate 3: Chromosomal abnormalities in cultured cells of *Andrographis paniculata* (L.)

a. Clumping and grouping of chromosomes. **b.** Sticky chromosomes. **c.** Broken bridge **d.** chromosome bridge**. e.** Chromosome fragments. **f.** Micronucleus formation. **g.** Many micronuclei formation.

Growth environments *in vitro* could possibly is responsible to enhance the occurrence of more polyploid cells, in *in-vitro* cultured cells due to the endoreduplication process that occurred occasionally within the population of cells.[14] Other factors include nuclear restitution or nuclear fragmentation caused by abnormalities such as lagging chromosomes and multipolar spindle that often result in binucleate or multinucleate cells as well as the occurrence of aneuploidy and reduced chromosome numbers. According to D'Amato,[15] the balance of auxin and cytokinin in the culture or induction media also influence the occurrence of nuclear fragmentation. In the present study, it is possible that abnormalities are caused by nuclear restitution due to abnormal mitoses and chromosomal arrest at the anaphase stage. Bayliss[16] have reported the supportive evidences while working on *Dianthus Caryophyllus* under *in vivo* and *in vitro* condition.

CONCLUSION

The *Andrographis paniculata* is medicinally important plant; research has confirmed that this herb has many pharmacological benefits including potent anti-inflammatory, anti-bacterial, anti-viral effects. In addition scientists have discovered that *Andrographis paniculata* boost the immune system, protects against cancer, prevents blood clots and maintains efficient digestive functioning. Our main objective was to trace out the possibility of variations at the cytological and protein banding pattern level in callus cells and normal grown cells.

The selected experimental system responds very positively for the induction of callus in MS media supplemented with hormonal concentration 2-4-D: BAP (1:0.5) with pH 5.8 and 0.8% agar. Our findings indicated variation in protein content, protein electrophoretic profiling (band numbers and RF ranges) in callus cells where compared with *in vivo* cells.

The results obtained on cell cytology of cultured cells indicated the evidences of structural chromosomal changes and grouping of chromosomes. Such anomalies are expected to induce variations at biochemical level too. Therefore the present work provides an insight to know the possible impact of cultured conditions on cytogenetical and biochemical components.

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Corresponding Author's Biography : Professor Vijay D. Mendhulkar

Professor and Head, Department of Botany. Institute of Science, 15-Madam Cama Road, Mumbai-400032. PG Teaching and Research Experience-20 years**. Recognized** Ph. D. Guide for The subject of Botany and Biotechnology (University of Mumbai). Research Area_-- Cytology and Genetics, Chemical Mutagenesis, Tissue Culture, Elicitation of Secondary Metabolites, In Vitro Mutagenesis and Nanobiotechnology. Publications-40. Googlesite-*https://sites.google.com/site/profvijaydmendhulkar*