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Cytotoxic and Genotoxic Assessment of Andrographis paniculata in the Allium cepa Test

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Abstract

Andrographis paniculata (Kalmegh) is a medicinal herb widely used in traditional Indian and Chinese systems of medicine. The major bioactive compound, andrographolide, possesses anti-inflammatory, hepatoprotective, antimicrobial, and immunomodulatory properties. Despite its therapeutic significance, evaluation of its cytotoxic and genotoxic potential is essential to ensure safety.

The present study aimed to assess the cytotoxic and genotoxic effects of leaf extracts of A. paniculata using the Allium cepa assay. Methanol, cold water, and hot water extracts (50% and 100%) were tested. Parameters evaluated included root induction, root length, mitotic index (MI), mitotic inhibition, and chromosomal aberrations.

Results indicated no significant inhibition of root growth or mitotic activity. Mild mitotic inhibition (2.31–3.90%) was observed but was not concentration dependent. Sticky chromosomes at metaphase were the only chromosomal aberration detected.

The findings suggest that A. paniculata extracts exhibit no significant cytotoxic or mitodepressive effects in the plant test system, although mild genotoxic stress was observed. Further studies using animal models are recommended to confirm genetic safety.

Keywords: *Andrographis paniculata, cytotoxicity, genotoxicity, Allium cepa, mitotic index, chromosomal aberration*

1. Introduction

Medicinal plants represent an important source of bioactive compounds used in traditional and modern medicine. India harbors approximately one-fourth of the world's medicinal plant diversity, with nearly 1,000 species used in Ayurveda, Siddha, and Unani systems. Among these, *Andrographis paniculata* (Family: Acanthaceae), commonly known as Kalmegh, is widely recognized for its therapeutic applications.

The plant is an erect annual herb (0.3–0.9 m tall) with quadrangular stems, opposite lanceolate leaves, and small white flowers marked with purple spots. It grows abundantly in Southeast Asia and is extensively cultivated in India, China, and Thailand.

The principal bioactive compound, andrographolide, is a diterpene lactone responsible for its anti-inflammatory, hepatoprotective, immunostimulatory, and antimicrobial activities. Other phytoconstituents include 14-deoxy-11,12-didehydroandrographolide, homoandrographolide, andrographan, andrographosterin, and stigmasterol.

Although the plant is widely consumed for therapeutic purposes, evaluation of its cytotoxic and genotoxic potential is essential to ensure safe use. The *Allium cepa* assay is a well-established model for screening cytotoxicity and chromosomal aberrations due to its sensitivity, reliability, and correlation with mammalian test systems.

Therefore, the present study was undertaken to investigate the cytotoxic and genotoxic effects of *A. paniculata* leaf extracts using the *Allium cepa* test system.

2. Materials and Methods

2.1 Plant Materials

Fresh leaves of *Andrographis paniculata* were collected and shade-dried. Healthy onion bulbs (*Allium cepa*) of uniform size were used for cytological analysis.

2.2 Preparation of Extracts

The dried leaves were powdered and used for extract preparation.

Methanol Extract:

50 g of powdered material was soaked in methanol for 72 hours. The extract was filtered and evaporated. The residue was dissolved in distilled water to obtain 100% concentration and diluted to prepare 50% concentration.

Cold Water Extract:

50 g powder was soaked in distilled water for 72 hours and filtered.

Hot Water Extract:

Prepared similarly using hot distilled water.

2.3 *Allium cepa* Assay

The outer scales of onion bulbs were removed carefully without damaging the root primordia. Bulbs were placed in:

- Cold water extract (50%, 100%)
- Hot water extract (50%, 100%)
- Methanol extract (50%, 100%)
- Tap water (control)

After 48 hours:

- Root number and root length were measured.
- Root tips were fixed in aceto-alcohol (1:3) for 24 hours.
- Hydrolyzed in 1 N HCl.
- Squash preparations were made using 1% acetocarmine.
- Five slides per treatment were analyzed.

2.4 Cytological Parameters

Mitotic Index (MI):

$$MI = \frac{\text{Number of dividing cells}}{\text{Total cells observed}}$$

Mitotic Inhibition (%):

$$\frac{MI_{control} - MI_{treated}}{MI_{control}} \times 100$$

Aberrant Cell Frequency (%):

$$\frac{\text{Aberrant cells}}{\text{Total cells observed}} \times 100$$

2.5 Statistical Analysis

Mean and standard error (SE) were calculated:

$$\sigma = \sqrt{\frac{\sum(X - \bar{X})^2}{N}}$$

$$SE = \frac{\sigma}{\sqrt{N}}$$

3. Results

No significant reduction in root induction or root length was observed. Root length ranged from 3.3–3.6 cm, and root number ranged from 8.3–9.6.

The mitotic index varied slightly between treatments (0.34–0.36), with the highest value recorded in 100% methanol extract. Mitotic inhibition ranged from 2.31–3.90% and was not concentration dependent.

Sticky chromosomes at metaphase were the only chromosomal aberration observed.

Table 1. Morphological and Cytological Effects of *A. paniculata* on *Allium cepa*

Parameter	Control	CW 50%	CW 100%	HW 50%	HW 100%	M 50%	M 100%
Root Length (cm)	3.6	3.6	3.3	3.6	3.6	3.3	3.6
Root Number (Mean ± SE)	9.3±0.19	8.6±0.38	9.3±0.38	8.3±0.19	9.3±0.19	9.0±0.38	9.6±0.19
Dividing Cells	109	110.4	111	112	108.8	108	112.2
Mitotic Index	0.34	0.35	0.35	0.34	0.35	0.34	0.36
Mitotic Inhibition (%)	0	2.31	2.93	2.94	2.96	3.30	3.90
Aberrant Cells (%)	0	2.6	3.0	4.0	3.0	3.3	2.4

Table 2. Frequency of Sticky Chromosomes

Treatment	1st MF	2nd MF	3rd MF	4th MF	5th MF	Mean ± SE
Control	0	0	0	0	0	0
Cold Water 50%	3	4	2	3	3	3.0±0.44
Cold Water 100%	5	6	7	5	5	5.6±0.16
Hot Water 50%	3	4	2	2	2	2.6±0.25
Hot Water 100%	5	6	6	6	5	5.6±0.15
Methanol 50%	3	3	4	4	4	3.6±0.15
Methanol 100%	6	6	7	5	6	6.0±0.28

4. Discussion

The present study demonstrated that *A. paniculata* extracts did not significantly inhibit root growth or mitotic activity in the *Allium cepa* test system. The mitotic index remained comparable to control values, indicating absence of strong cytotoxic or mitodepressive effects.

Mild mitotic inhibition observed was not dose dependent, suggesting limited interference with DNA synthesis or spindle formation. Strong cytotoxic agents typically produce marked reduction in mitotic index and concentration-dependent inhibition, which was not observed here.

Sticky chromosome formation was the only genotoxic manifestation detected. Chromosomal stickiness may result from disturbances in chromatin organization, DNA-protein crosslinking, or partial depolymerization of nucleoproteins. However, the absence of severe aberrations such as bridges, laggards, or C-metaphase indicates that the extracts do not cause major structural chromosomal damage.

Methanol extract showed slightly higher stickiness frequency, possibly due to greater extraction efficiency of bioactive phytochemicals. Overall, the findings suggest minimal genotoxic stress under the tested conditions.

5. Conclusion

The present investigation concludes that:

- *Andrographis paniculata* extracts do not significantly inhibit root growth or mitotic activity in *Allium cepa*.
- Mitotic inhibition observed was mild and non–dose dependent.
- Sticky chromosomes were the only chromosomal aberration recorded.
- No severe structural chromosomal abnormalities were detected.

Thus, under experimental conditions, *A. paniculata* appears to have no significant cytotoxic or mitodepressive effect in the plant model system. However, mild chromosomal stickiness indicates the need for further investigation using mammalian systems to fully confirm genetic safety.

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