Comparative in vitro cytotoxicity of aqueous and ethanolic extracts of *Andrographis paniculata* in Vero and BHK-21 cell lines

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Abstract

The present study illustrates the difference in the *in vitro* cytotoxicity of aqueous and ethanolic extracts of *Andrographis paniculata* whole plant in Vero and Baby Hamster Kidney-21 (BHK- 21) cells. The cells were treated with two fold serial dilutions of the aqueous and ethanolic extracts ranging from 3200 to 3.12µg/ml for 48 hours. The MTT assay was performed to determine the cell viability after treating with extracts. The IC_{50} of andrographolide (98%), aqueous and ethanolic extracts in Vero cells were found to be 400, 3200 and 1200 µg/ml respectively. The IC_{50} of andrographolide, aqueous and ethanolic extracts in BHK-21 cells were 5.125, 400 and 1.56 µg/ml respectively. The findings of the study demonstrate that among the three treatments, andrographolide in its pure form was more toxic to both the cell lines as opposed to the whole plant extracts. Further, Vero cells could tolerate higher concentration of both types of extracts compared to BHK-21 cells, implying the tolerance level to different extracts being determined by the cell type.

Key words: *Andrographis paniculata*, andrographolide, aqueous extract, ethanolic extract, cytotoxicity, *in vitro,* cell line

Introduction

Andrographis paniculata is an annual, herbaceous plant belonging to the family *Acanthaceae* and it is commonly called as "king of bitters". It has abundantly been cultivated in different parts of the world such as India, Sri Lanka, Indonesia and Malaysia (Hossain *et al*., 2014) for various medicinal applications. The existing evidence suggests that components present in *A. paniculata* exhibit a range of beneficial pharmacological properties, including antityphoid, antifungal, antihepatotoxic, antibiotic, antimalarial, anti-hepatitis, anti-thrombogenic, anti-inflammatory, immunomodulatory and antitumor activities (Dai *et al*., 2019). We have previously reported the presence of forty-one and forty-four different components in aqueous and ethanolic extracts of *A. paniculata* respectively and including phenols, tannins, hydrolysable tannins, saponins, flavonoids and terpenoids in both the extracts. Additionally, cardiac glycosides, phlobatannins were present exclusively in the ethanolic and aqueous extract respectively (Navadeepa *et al.,* 2024). Andrographolide is a bicyclic diterpenoid lactone, the main active ingredient isolated from the herb *Andrographis paniculata* that displays diverse biological effects including anti-viral, anti-inflammatory, anti-apoptotic, and anti-hyperglycemic properties (Burgos *et al.,* 2020). The present study aims to evaluate the difference in the cytotoxicity/viability of Vero and BHK- 21cells to andrographolide (98%), aqueous and ethanolic whole plant extracts of *A. paniculata* that would be helpful for the researchers to choose the non-cytotoxic dose before conducting any *in vitro* studies.

Materials and Methods

Collection of plant and preparation extract

The whole dried plant of *Andrographis paniculata* (Burm.f.) Wall. ex Nees were collected from the Siddha medicinal plant garden, Mettur, Tamil Nadu, India and authentication of the plant was done at Botanical Survey of India, Coimbatore, Tamil Nadu (No. BSI/SRC/5/23/2023-24/Tech-107 dated 19.02.2024). The whole plant was cut into pieces and was ground into a fine powder using electric blender. the obtained powder was stored in an air tight container until further use.

Preparation of aqueous and ethanolic extracts of *A. paniculata*

"A 10% (w/v) aqueous extract and a 10% (w/v) ethanolic extract of *A. paniculata* whole plant (APWP) were prepared by adding 20 g of dry powder to 200 ml of distilled water and 20 g of dry powder to 200 ml of 70 per cent ethanol respectively. The mixture was kept under magnetic stirring for 48 h and filtered through Whatman No.1 filter paper. The filtrate was collected and dried by incubating at 42℃ for 48 h in a petri dish to evaporate the solvent. The dried extract powder was collected, measured and stored in an air tight container until further use (Nagajothi *et al*., 2018).

Preparation of serial dilutions of the extracts and andrographolide

The aqueous and ethanolic extracts and andrographolide (98%) [Cat. No, 365645-Sigma] were weighed and dissolved in the DMEM media starting from 3200 µg/ml to 1.56 µg per ml working dilutions. The two fold dilutions of the extracts were prepared from the stock solution as mentioned below.

Determination of Cytotoxicity by MTT assay

Immortalized adherent cells, Vero (P-87) and BHK-21(P-72) cells were grown in T25 cell culture flasks in DMEM cell culture medium supplemented with 10% FBS, 1 per cent antibiotic, antimycotic solution and 1% HEPES and incubated at 37ºC to form a confluent monolayer. The monolayer was trypsinized and seeded at 5×10^4 cells/well in 96 well microplates, incubated for 12 h at 37°C. Then the growth medium was removed and fresh DMEM supplemented with 2 per cent FBS containing different concentrations of the APWP extracts and andrographolide (pure) were added in triplicate and duplicate respectively to the wells of each concentration and along the DMSO treated control cells and incubated for 48 h. Later, 10 µl of filter sterilized 0.005 per cent MTT solution was added to each well and incubated at 37°C for 4 hours. Then the media from the wells was removed without disturbing the MTT crystals and 100 µl of DMSO was added to the wells to dissolve dark blue formazan crystals and mixed thoroughly. The plates were incubated at room temperature for 1 h ensuring that all crystals were dissolved and mixed again before readings were taken on spectrophotometer at a wavelength of 570 nm and 630 nm.

The percentage of live cells were calculated from the OD value of each treatment using the formula (Sirakanyan *et al*., 2021):

$$\text{Cell viability percentage (\%)} = (\text{ODT}/\text{ODC}) \times 100$$

where ODT was the average optical density value of treated and ODC was the average OD value of control cells. The IC_{50} value was defined as the concentration that reduced cell viability by 50% relative to untreated controls.

Results and Discussion

The extracts of the plants are being explored to evaluate their diverse biological and medicinal properties by the researcher initially under *in vitro* settings before experimenting in a laboratory animal model or in the animal species of interest. The cytotoxicity of the extracts should be ascertained before proceeding for any experiment. This study compared the cytotoxic activities of andrographolide, aqueous and ethanolic extracts of *A. paniculata* whole plant (APWP) in Vero and BHK-21 cells under *in vitro* conditions and illustrate the difference in the *in vitro* cytotoxic effects in a dose-dependent manner.

In the present study, the IC_{50} dose of andrographolide was determined as 400µg/mL in Vero cells (Fig.1) and 5.125 µg/mL in BHK-21 cells (Fig.2) at 48 h. Kandanur *et al*. (2019) and Theerawatanasirikul *et al*. (2022) determined that IC_{50} of andrographolide in BHK-21 cells as > 250 µM and 116.8 ± 0.03 µM respectively. Banerjee *et al.,* (2016) reported that the IC_{50} of andrographolide in the breast cancer cell lines at 24 and 48 h was found to be 51.98 µM and 30.28 µM respectively in MDA-MB-231 and 61.11 µM (24 h) and 36.9 µM (48 h) respectively in the MCF-7 cells. Whereas, the IC_{50} was estimated to be 137.9 µM (24 h) and 106.1 µM (48 h) for normal human mammary epithelial cells, MCF-10A signifying the IC_{50} at 48 h was nearly 3.5 fold higher than that for MDA-MB-231 cells. Further, Goel *et al.,* (2021) reported the IC_{50} of andrographolide as 42.723 ± 0.668 µg/ ml in HCT 116 cells. These results indicate a considerable difference in the sensitivity of different types of cells to andrographolide and further emphasize that healthy cells were more tolerant to higher concentrations than that of cell line.

The IC_{50} dose of aqueous extracts of APWP at 48 hrs was determined as 3200µg/mL in Vero cells (Fig.1) and 400µg/mL in BHK-21 cells (Fig.2) in the current study. Sangeetha *et al*. (2015), Sa-Ngiamsuntorn *et al*. (2021) and Nagajothi *et al*. (2023) mentioned that the non-cytotoxic dose of aqueous extract of *A. paniculata* in Vero cells as 31.25/ml, 100 µg/ml and >25µg/ml respectively. Ala *et al.* (2018) reported that the cell viability of the splenocytes and thymocytes and hepatocytes with aqueous extracts of *A. paniculata* at the highest concentration (500µg/mL) and lowest concentration (4µg/mL) were between 85 and 91 per cent and 85 and 94 per cent respectively which were lower than the untreated cells showing viability percentage of 86–95%. In contrast to the earlier reports, the IC_{50} dose of aqueous extracts of APWP in the present study is comparatively higher. However, the aqueous extracts at all concentrations exhibited maximum cell viability percentage and lowest cytotoxicity at 48h incubation in both the cell lines compared to the ethanolic extracts and androgrpholide.

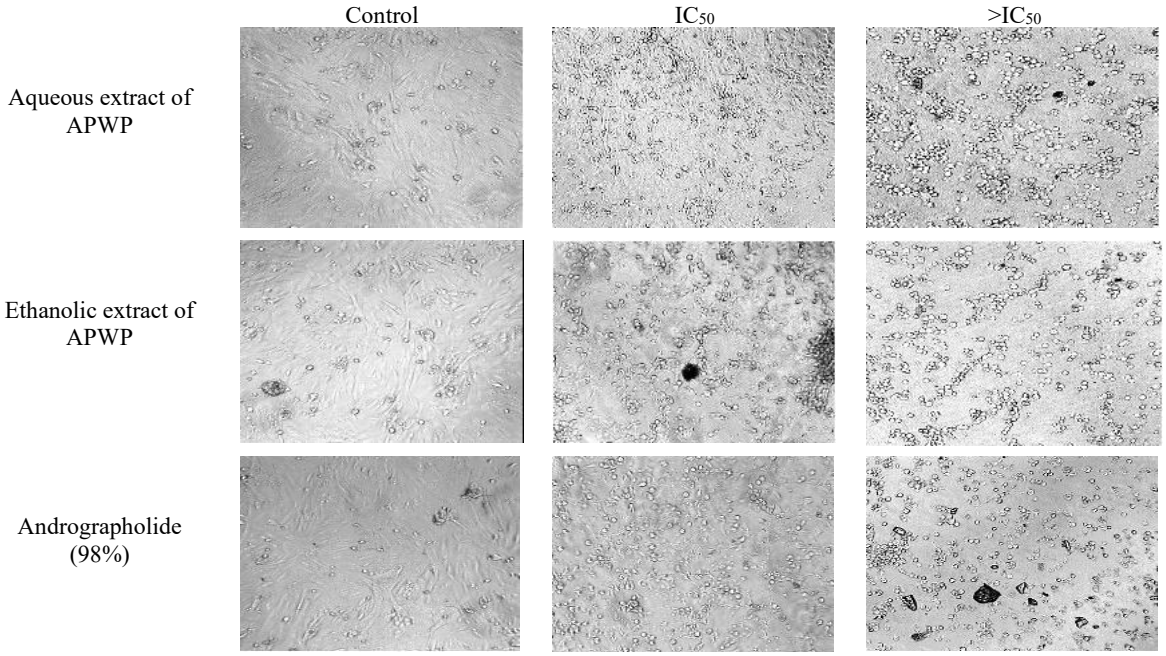


Fig 1: Morphological changes in Vero cells treated with andrographolide, aqueous and ethanolic APWP extracts at 48 h

The IC_{50} dose of ethanolic extracts of APWP at 48 h was determined as 1200µg/mL in Vero cells **(Fig.1 & 3)** and 1.56 µg/mL in BHK-21 cells **(Fig.2 & 4)**. Ramalingam *et al*. (2018), Jain *et al*. (2020), Therasa *et al*. (2020), Abdullahi *et al*. (2020) and Paul *et al*. (2021) mentioned that the non-cytotoxic dose of ethanolic extract of *A. paniculata* in Vero cells as ≤500, 12.5, 250, 142 and 250 µg/ml. The IC_{50} of methanolic extract of *A. paniculata* leaf in IMR-32 and HT-29 cell lines were reported to be 200 µg/ml. Similarly, Goel *et al.,* (2021) reported that the IC_{50} of the methanolic extracts of *A. paniculata* in Human Colorectal 116 (HCT 116) cells were 45.32 ± 0.86 µg/ ml, whereas, in HepG2 cells as 60.32 ± 1.05 µg/ml and did not show significant cytotoxicity in HEK 293 cell line (human embryonic kidney cell) up to 120 µg/ml concentration.

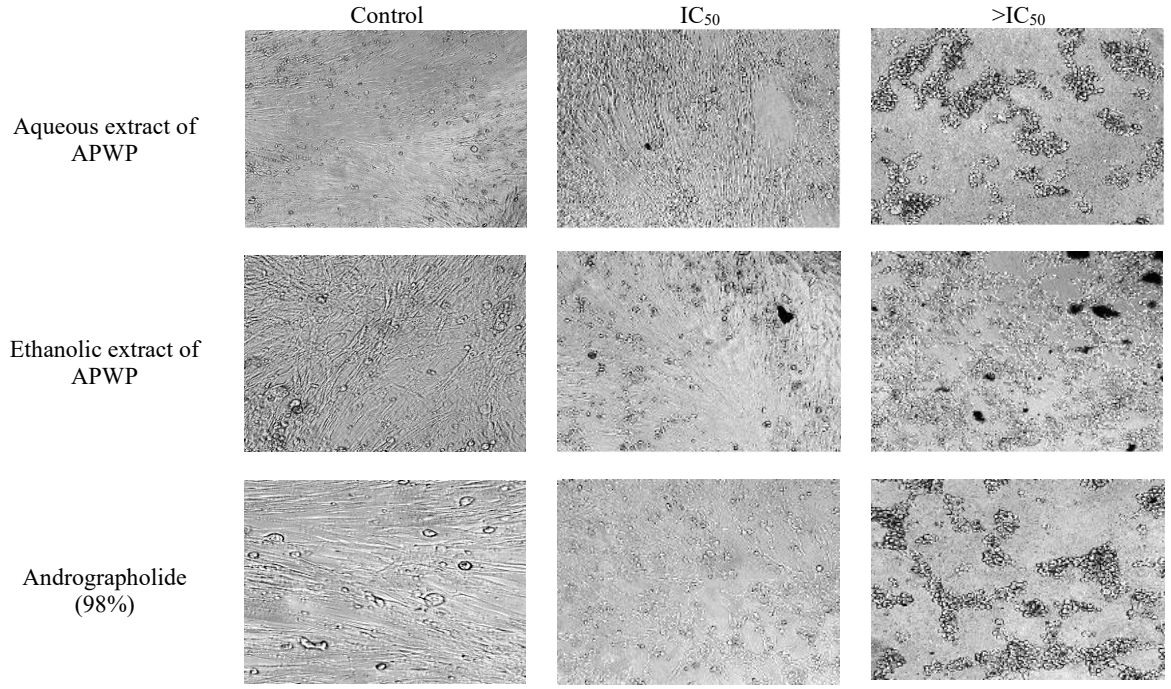


Fig 2: Morphological changes of BHK-21 cells treated with Andrographolide, aqueous and ethanolic APWP extracts at 48 h

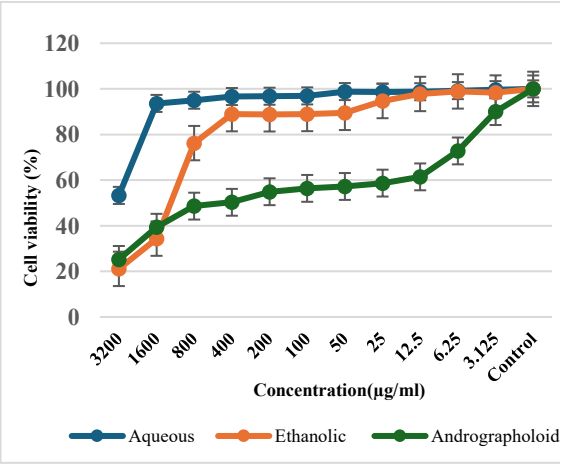


Fig 3: Chart showing cell viability percentage of Andrographolide, aqueous and ethanolic extracts of APWP in Vero cells at 48 h

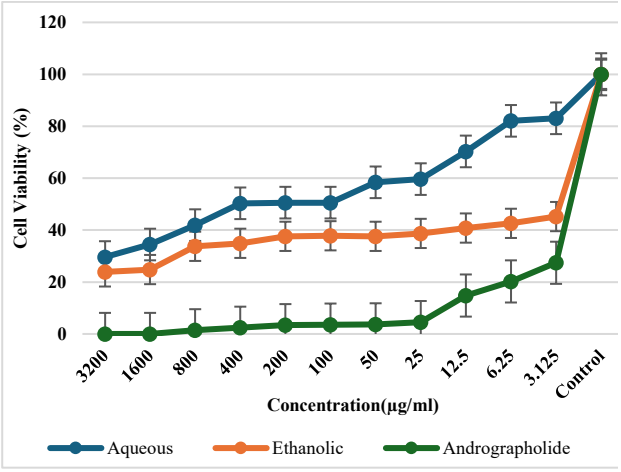


Fig 4: Chart showing cell viability percentage of Andrographolide, aqueous and ethanolic extracts of APWP in BHK-21 cells at 48 h

The findings of the study demonstrate Vero cells could tolerate higher concentration of both types of extracts in comparison with BHK cells implying the tolerance level being determined by the cell type. Interestingly, the aqueous extract of *A. paniculata* produce less cytotoxicity even at higher concentrations as compared to the ethanolic counterpart signifying the method of extraction influencing the release of

phytochemical components with cytotoxic potential. The cytotoxicity of the ethanolic extract producing 50% cell death with much lesser concentration in both the cell types could be attributed to the source of the plant and its geographical conditions in which the plants are grown influencing the phytochemical components and concentration of cytotoxic components present in them. Surprisingly, similar pattern of the cytotoxicity with andrographolide was observed in Vero cells and BHK cells. The andrographolide in its pure form (98%) are found to be highly toxic as compared to the crude extracts evaluated in this study as well the erstwhile studies conducted by several authors.

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References

1) Abdullahi, S. A., Unyah, N. Z., Nordin, N., Nasiru, W. M., Alapid, A., Basir, R., and Abd Majid, R. (2020). "Evaluation of anti-parasitic effect of *Andrographis paniculata* (burm. f) nees, ethanolic extract on *Toxoplasma gondii*". Southeast Asian Journal of Tropical. Medicine and Public Health., 51(5): 675-685.
2) Ala AA, Olotu BB, Ohia CMD (2018). Assessment of cytotoxicity of leaf extracts of *Andrographis paniculata* and *Aspilia africana* on murine cells *in vitro*. Archives of Basic and Applied Medicine, 6(1), 61–65. PMID: 30234147
3) Banerjee, M., Chattopadhyay, S., Choudhuri, T., Bera, R., Kumar, S., Chakraborty, B., and Mukherjee S.M (2016). Cytotoxicity and cell cycle arrest induced by andrographolide lead to programmed cell death of MDA-MB-231 breast cancer cell line. Journal of Biomedical Science, 23, 40 DOI: 10.1186/s12929-016-0257-0
4) Burgos, R.A, Alarcón., P, Quiroga, J., Manosalva, C., Hancke, J. (2020) Andrographolide, an Anti-Inflammatory Multitarget Drug: All Roads Lead to Cellular Metabolism. Molecules. 26(1):5. DOI: 10.3390/molecules26010005.
5) Dai, Y., Chen, S. R., Chai, L., Zhao, J., Wang, Y., and Wang, Y. (2019). "Overview of pharmacological activities of *Andrographis paniculata* and its major compound andrographolide". Critical reviews in food science and nutrition, 59(sup1), S17–S29. https://doi.org/10.1080/10408398.2018.1501657
6) Goel N, Gajbhiye RL, Saha M, Nagendra C, Reddy AM, Ravichandiran V, Das Saha K, Jaisankar P (2021). A comparative assessment of *in vitro* cytotoxic activity and phytochemical profiling of *Andrographis nallamalayana* J.L. Ellis and *Andrographis paniculata* (Burm. f.) Nees using UPLC-QTOF-MS/MS approach. RSC Advances. 11(57):35918-35936. DOI: 10.1039/d1ra07496b.
7) Hossain, M. S., Urbi, Z., Sule, A., & Hafizur Rahman, K. M. (2014). Andrographis paniculata (Burm. f.) Wall. ex Nees: a review of ethnobotany, phytochemistry, and pharmacology. The Scientific World Journal, 2014, 274905. https://doi.org/10.1155/2014/274905
8) Jain, J., Kumar, A., Narayanan, V., Ramaswamy, R. S., Sathiyarajeswaran, P., Devi, M. S., and Sunil, S. (2020). "Antiviral activity of ethanolic extract of Nilavembu Kudineer against dengue and chikungunya virus through in vitro evaluation". Journal of Ayurveda and Integrative Medicine, 11(3), 329–335. DOI: 10.1016/j.jaim.2018.05.006.
9) Kandanur, S. G. S., Tamang, N., Golakoti, N. R., & Nanduri, S. (2019). "Andrographolide: A natural product template for the generation of structurally and biologically diverse diterpenes". *European Journal of Medicinal Chemistry*, 176: 513-533. DOI: 10.1016/j.ejmech.2019.05.022
10) Nagajothi S, Mekala P, Raja A, Raja MJ, and Senthilkumar P. (2018). "*Andrographis paniculata*: Qualitative and quantitative phytochemical analysis". Journal of Pharmacognosy and Phytochemistry., **7(4):**1251-1253.
11) Nagajothi, S., P.Mekala, A.Raja, M.J.Raja, P.Senthil Kumar and G.Thirumalaisamy (2023). Antiviral activity of *Andrographis paniculata* against NDV2K35 strain of Newcastle disease virus in chicken embryo fibroblast cells. The Pharma Innovation Journal, 12(4): 1461-1469.
12) Navadeepa T, Ramya K, Sukumar K, Sasikala M (2024). Phytochemical screening and GC-MS analysis of whole plant extract of *Andrographis paniculata*. International Journal of Veterinary Sciences and Animal Husbandry; 9(6S)**:**249-254. https://www.doi.org/10.22271/veterinary
13) Paul, A., Vibhuti, A., and Raj, V. S. (2021). "Evaluation of antiviral activity of *Andrographis paniculata* and *Tinospora cordifolia* using *in silico* and *in vitro* assay against DENV-2". Journal of Pharmacognosy and Phytochemistry, 10(2): 486-496. DOI: 10.22271/phyto.2021.v10.i2f.13847
14) Ramalingam, S., Karupannan, S., Padmanaban, P., Vijayan, S., Sheriff, K., Palani, G., & Krishnasamy, K. K. (2018). "Anti-dengue activity of Andrographis paniculata extracts and quantification of dengue viral inhibition by SYBR green reverse transcription polymerase chain reaction". Ayu, 39(2), 87–91. DOI: 10.4103/ayu.AYU_144_17

15) Sangeetha, K., and S. Rajarajan. (2015). "*In-vitro* antiviral activity of Indian medicinal plants to Asian and East Central South African lineage of Chikungunya virus". International Journal of Pharmaceutical Sciences and Research (IJPSR)., 6(2):692 ISSN (Electronic): 0975-8232
16) Sa-Ngiamsuntorn, K., Suksatu, A., Pewkliang, Y., Thongsri, P., Kanjanasirirat, P., Manopwisedjaroen, S., and Hongeng, S. (2021). "Anti-SARS-CoV-2 activity of *Andrographis paniculata* extract and its major component andrographolide in human lung epithelial cells and cytotoxicity evaluation in major organ cell representatives". Journal of Natural Products, 84(4), 1261–1270. DOI: 10.1021/acs.jnatprod.0c01324
17) Sirakanyan, S., Arabyan, E., Hakobyan, A., Hakobyan, T., Chilingaryan, G., Sahakyan, H., & Zakaryan, H. (2021). 'A new microtubule-stabilizing agent shows potent antiviral effects against African swine fever virus with no cytotoxicity". Emerging Microbes and Infections, 10(1): 783-796.
18) Theerawatanasirikul S, Lueangaramkul V, Thangthamniyom N, Chankeeree P, Semkum P, Lekcharoensuk P (2022). Andrographolide and Deoxyandrographolide Inhibit Protease and IFN-Antagonist Activities of Foot and Mouth Disease Virus $3C^{pro}$. Animals (Basel). *Animals*, 12(15): DOI: 10.3390/ani12151995.
19) Therasa, S. A., G. Sobiya and S.M. Parimala. (2020). "Leaves of *Andrographis paniculata* is an antioxidant and anticancer agent". *20.* Asian Journal of Pharmaceutical and Clinical Research*.,* 13(8): 213-217 DOI: 10.22159/ajpcr.2020.v13i8.37014