Biocatalysis and Agricultural Biotechnology 22 (2019) 101381

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: http://www.elsevier.com/locate/bab

Cytotoxic effect induced apoptosis in lung cancer cell line on *Ageratina adenophora* leaf extract

Suganya Mani [a], Karthik Natesan [b], Kavitha Shivaji [a], Mythili Gnanamangai Balasubramanian [a], Ponmurugan Ponnusamy [b,*]

[a] Department of Biotechnology, K. S. Rangasamy College of Technology, Tiruchengode, 637 215, Tamil Nadu, India
[b] Department of Botany, Bharathiar University, Coimbatore, 641 046, Tamil Nadu, India

ARTICLE INFO

Keywords:
Anticancer activity
MTT assay
Giemsa staining
Propidium iodide staining

ABSTRACT

Ageratina, adenophora, (A. adenophora) is a conventional prescription plant utilized for various purposes in around the world. However, its biological impacts and their potential components have not been adequately examined. The present investigation assessed the anticancerous properties of *A. adenophora* leaf extract. The methanolic extract inspired the most pronounced cytotoxic effect in lung cancer cells (A549), as appraised by MTT assay performed 24 h after treatment, demonstrating an IC_{50} estimation of 50.08 ± 0.14 of low concentration in 40 μg/mL. The anti-proliferative movement watched following *A. adenophora* treatment was related to morphological (Giemsa staining) and nuclear (Propidium Iodide staining) changes of apoptosis. The outcomes propose that *A. adenophora* could present as a potential wellspring of alternative medication drugs for treating cancer. Further research is required to find out the effective mechanisms responsible for anticancer properties.

1. Introduction

Cancer is a horrible illness, establishing an abnormal growth and multiplication of malignant cells in the body (Pragya and Khan, 2016). Malignant growth is a noteworthy general medical issue and its leading causes of death around in the world (Randhawa and Alghamdi, 2011; Lu et al., 2009). The clinical trial of malignant growth was analyzed by different medicines like radiation, chemotherapy and surgery (Kishi et al., 2011; Wesselborg et al., 2011). Be that as it may, chemotherapeutic medications are profoundly dangerous and have severe side effects (Tavaresa et al., 2010). Accordingly, the few phytochemicals are available in the herbal products and plants may can possibly go about as preventive or restorative agents against different human tumors (Gupta et al., 2004; Bharath et al., 2017). Currently the researchers have mainly focused on the medicinal plant therapies in day by day and also pharmaceutical industries targeted natural products from plant derived compounds with anticancer drugs.

Ageratina adenophora is a plant from the Asteraceae family, which contain a rich measure of flavonoids. Antioxidant and antimicrobial properties have been accounted for in already (Harish Kumar et al., 2014). It is accounted for to have different therapeutic properties and discover the conventional medications (Kurade et al., 2010). *A. adenophora* leaf used to treat the different diseases, for example, wounds, a sleeping disorder, jaundice and ulcers (Subba and Kandel, 2012). Until this point, there have been no effective mediation produced for anticancer drugs. The present study investigated to find out the anticancer activities of *A. adenophora* leaf extract against lung cancer (A549) cell line.

2. Results and discussion

2.1. Cytotoxicity assay

The cytotoxic effect of *A. adenophora* extract against A549 cells with increasing concentrations of (20–100 μg/mL) for 24 h, which was confirmed by MTT assay. The outcomes demonstrated that methanolic extract of *A. adenophora* significantly induce cytotoxicity in a dose dependent manner. *A. adenophora* displayed the strong antiproliferative activity with IC_{50} values of 60.13 ± 0.11, 50.08 ± 0.14, and 43.28 ± 0.27 with the concentrations of 20, 40 and 60 μg/mL against A549 cells respectively was appeared in Fig. 1. The cell viability was gradually decreased, when treated with low concentration of 40 μg/mL. Accordingly, these IC_{50} focuses (20, 40 and 60 μg/mL) of the *A. adenophora* were utilized for future analyses. MTT examines being the

* Corresponding author.
E-mail address: cancerbiology41@gmail.com (P. Ponnusamy).

https://doi.org/10.1016/j.bcab.2019.101381
Received 10 September 2019; Received in revised form 24 September 2019; Accepted 8 October 2019
Available online 12 October 2019

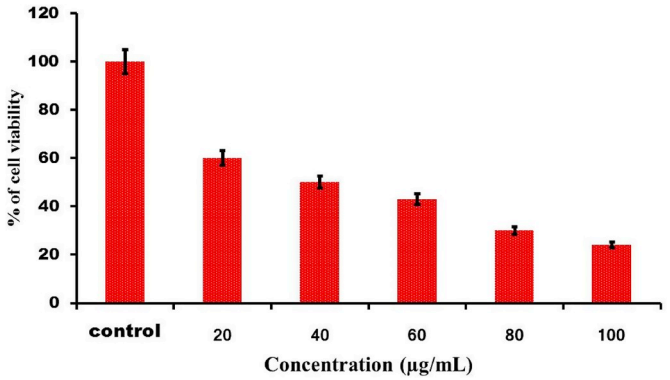

Fig. 1. Cytotoxic (MTTassay) effect of *A. adenophora* leaf extract on A549 cells for 24 h. Data are represented in the mean ± standard error.

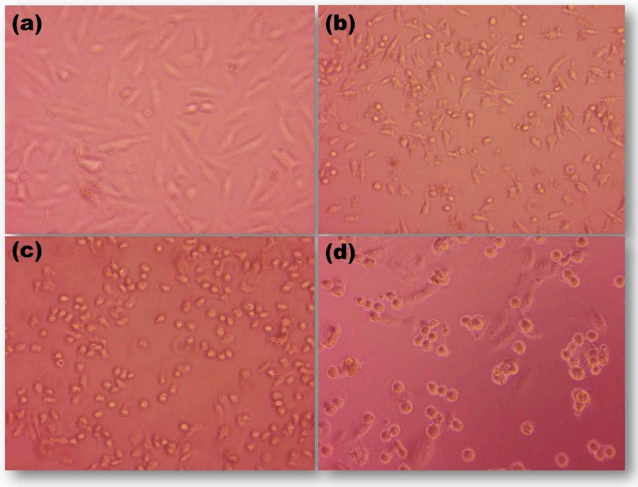

Fig. 2. Phase – contrast microscopy image of A549 cells treated with *A. adenophora* leaf extract for 24 h. (a) Control, (b) 20 µg/mL, (c) 40 µg/ml (d) 60 µg/ml.

most sensitive in identifying cytotoxic effect, in view of mitochondrial respiratory action, would offer early signs of harmfulness following exposure to a mitochondrial toxicant (Fatma et al., 2012).

2.2. Morphological observation

The apoptotic effect of the *A. adenophora* extract on A549 cells was pictured under an optical-inverted light microscopy. The cells were treated with IC_{50} concentrations which were chosen based on the MTT assay and incubated for 24 h. A549 cells are polygonal or unpredictable in shape and grown in a tightly connected manner. Untreated A549 cells demonstrated that the cells maintained their unique morphology, structure containing a few nucleoli and showed ordinary, healthy round shape and a predictable size, with smooth cell membrane as appeared in Fig. 2. On the other hand, extract-treated A549 cells showed cell shrinkage, chromatin condensation, nuclear fragmentation and cells in round shape, it confirms the depletion of apoptosis. It was clearly differentiated with cellular morphological characteristics, which was typically indicating of programmed cell death occurred in cancer cells (Du et al., 2012).

2.3. Giemsa staining

In the present study, apoptosis was also assayed cytologically using the Giemsa staining. Morphological changes in cell nuclei were

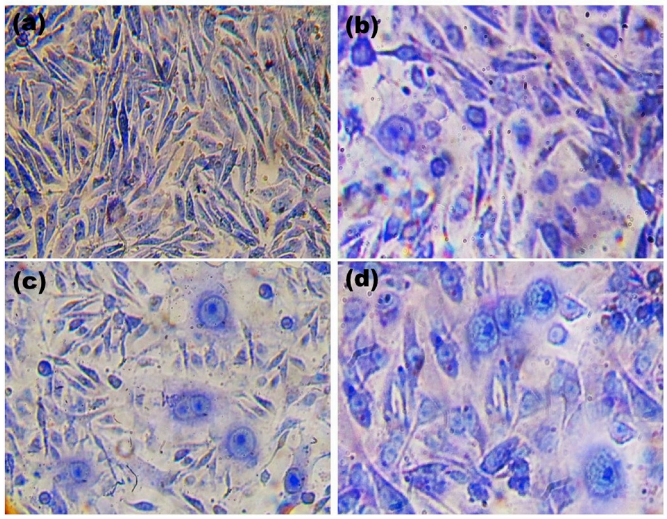

Fig. 3. Morphological changes of *A. adenophora* leaf extract on A549 cells stained with Giemsa staining. (a) Morphology of A549 cells without treatment (control), (b) 20 µg/mL, (c) 40 µg/ml (d) 60 µg/ml.

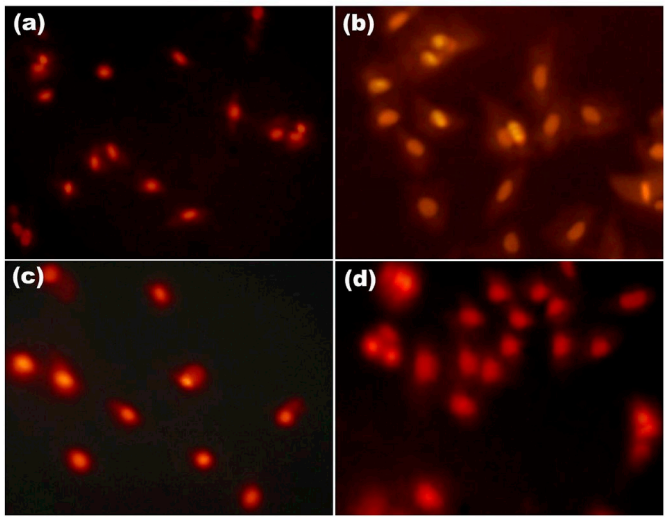

Fig. 4. Nuclear changes of *A. adenophora* leaf extract on A549 cells stained with Propidium Iodide staining. Untreated cells, (b) 20 µg/mL, (c) 40 µg/ml (d) 60 µg/ml.

determined by fluorescence microscopy. Giemsa staining was used to evaluate the apoptosis of cell death in A549 cells, *A. adenophora* extract-treated with IC_{50} concentrations of 20, 40 and 60 µg/mL for 24 h. There was no morphological change found in the untreated control cells. At the same time cells were seen with progressively morphological changes such as cytoplasmic condensation, cell shrinkage, membrane blebbing as shown in Fig. 3. Helmy and Azim (2012) demonstrated that Cisplatin in various concentrations induced apoptosis in cultured Hep2 cells using Giemsa staining.

2.4. Propidium iodide staining

The apoptotic cells were detected using fluorescent microscope. The cells were treated with IC_{50} concentration (20, 40 and 60 µg/mL) of *A. adenophora* extract. The results are shown in Fig. 4. The apoptosis was noticed with the nuclear changes in the cell shape, chromatin condensation and nuclear fragmentation these nuclear changes are due to the activation of caspase cascades and cell death or apoptosis. When the

methanolic extract of *A. adenophora* was administrated to A549 cells, a steep increase in the number of apoptotic cells was observed. The ability of the *A. adenophora* to induce apoptosis was determined by propidium iodide staining (Paul et al., 2011).

3. Conclusion

In conclusion, the present study demonstrated that *A. adenophora* exhibits strong anticancer effects on lung cancer (A549) cell proliferation, and Giemsa and PI staining revealed the morphological and nuclear changes of *A. adenophora* extract induced cell apoptosis with chromatin condensation and membrane blebbing in A549 cells. This plant has more effective anticancer properties against lung cancer cells.

Declaration of competing interest

We author declare that we have no conflict of interest.

Acknowledgement

The authors gratefully acknowledge the DST–INSPIRE sponsored program, Department of Science and Technology, New Delhi (REF. NO: DST/INSPIRE Fellowship/2015/IF150459) and also thank the Department of Biotechnology, K. S. Rangasamy College of Technology for the support offered towards the study. The authors also acknowledge DST–FIST (fund for infrastructure for science and technology) FIST NO: 368 for the support given carry out the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101381.

References

Bharath, G., Swarna Latha, B., Alsharaeh, E.H., Prakash, P., Ponpandian, N., 2017. Enhanced hydroxyapatite nanorods formation on graphene oxide nanocomposite as a potential candidate for protein adsorption, pH controlled release and an effective drug delivery platform for cancer therapy. Anal. Methods 9, 240–252.

Du, G.J., Zhang, Z., Wen, X.D., Yu, C., Calway, T., Yuan, C.S., Wang, C.Z., 2012. Epigallocatechingallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. Nutrients 4, 1679–1691.

Fatma, S.D., Widurini, R., Farida, R., Irmaleny, 2012. *In vitro* cytotoxicity of *jatropha curcas* in epithelial and fibroblast cells. J. Nat. Prod. 5, 214–221.

Gupta, S., Zhang, D., Yi, J., Shao, J., 2004. Anticancer activities of Oldenlandia diffusa. J. Herb. Pharmacother. 4, 21–33.

Harish Kumar, K., Shanmugavadivu, M., Ranjithkumar, R., Selvam, K., 2014. Antibacterial activity of leaf extracts of Ageratina adenophora L medicinal plant of Nilgiris Hill, Tamilnadu against human pathogens. Int. J. Biosci. Nanosci. 1, 1–3.

Helmy, I.M., Azim, A.M.A., 2012. Efficacy of image in the assessment of apoptosis. Diagn. Pathol. 7, 15.

Kishi, S., Yang, W., Boureau, B., Morand, S., Das, S., Chen, P., Cook, E.H., Rosner, G.L., 2011. Effects of prednisone and genetic polymorphism on etoposide disposition in children with acute lymphoblastic leukemia. Blood J. 103, 67–72.

Kurade, N.P., Jaitak, V., Kaul, V.K., Sharma, P., 2010. Chemical composition and antibacterial activity of essential oils of *Lantana camara, atum Houstonianum* and *Eupatorium adenophorum*. Pharm. Biol. 48, 539–544.

Lu, Q.Y., Yang, Y., Jin, Y.S., Zhang, Z.F., Heber, D., Frederick, P., Li, Steven, M., Dubinett, Melissa, A., Sondej, Loo, J.A., Rao, J.Y., 2009. Effect of green tea extract on lung cancer A549 Cells: proteomic identification of proteins associated with cell migration. Proteomics 9, 757–767.

Paul, S., Bhattacharaya, S.S., Boujedaini, N., Bukhsh, A.R.K., 2011. Anticancer potentials of root extract of *Polygala senega* and its PLGA nanoparticles-encapsulated form. Evid. Based Complement Altern. Med. 5, 13–20.

Pragya, T., Khan, M.J., 2016. Molecular and computational studies on apoptotic pathway regulator, Bcl-2 gene from breast cancer cell line MCF-7. Indian J. Pharm. Sci. 78, 87–93.

Randhawa, M.A., Alghamdi, M.S., 2011. Anticancer activity of *Nigella sativa* (black seed) – a review. Am. J. Chin. Med. 39, 1075–1091.

Subba, B., Kandel, R.C., 2012. Chemical composition and bioactivity of essential oil of *Ageratina adenophora* from Bhaktapur District of Nepal. J. Nepal Chem. Soc. 30, 78–86.

Tavaresa, L., Fortalezasa, S., Carrilhoa, C., McDougallb, G.J., Stewartb, D., Ferreiraa, R. B., et al., 2010. Antioxidant and antiproliferative properties of strawberry tree tissues. J. Berry Res. 1, 3–12.

Wesselborg, S., Ingo, H.H., Rossmann, E., Los, M., Osthoff, K.S., 2011. Anticancer drugs induce caspase -8/FLICE activation and apoptosis in the absence of CD95 receptor/ligand interaction. Blood 93, 3053–3063.