SHORT COMMUNICATION Indian Journal of Animal Research, Volume 58 Issue 4: 695-697 (April 2024)

Comparative Evaluation of Apoptotic Effects of Vincristine, Cisplatin and Their Hydrogel Scaffolds on HeLa Cell Line

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10.18805/IJAR.B-4412

ABSTRACT

The present study has been undertaken for comparative evaluation of apoptotic effects of vincristine, cisplatin and their hydrogel scaffolds on HeLa cell line. Apoptosis can be visualized as a ladder pattern due to DNA cleavage by the activation of a nuclear endonuclease by standard agarose gel electrophoresis. Results showed the formation of the DNA ladder in gel electrophoresis by induction of apoptosis in HeLa cell line treated with 20 µg/ml of vincristine alone, 20 µg/ml cisplatin alone and their scaffolds, a typical ladder pattern of internucleosomal fragmentation was observed in cells after 24 hrs treatment. DNA ladder assay of genomic DNA from HeLa cells was isolated and subjected to electrophoresis in agarose gel (1.8%) and 1kb DNA ladder. DNA fragments were visualized under a UV trans-illuminator and compared with a standard marker. On the basis of the parameters studied, it was concluded that the formation of the DNA ladder in gel electrophoresis by induction of apoptosis in HeLa cell line treated with vincristine, cisplatin alone and their scaffolds showed best fragmentation in vincristine and cisplatin, however, their scaffolds are moderately effective when used in appropriate dose on HeLa cell line.

Key words: Cisplatin, HeLa cell line, Hydrogel scaffolds, Vincristine sulphate.

Vincristine sulphate belongs to a group of medications known as vinca alkaloids; a mitotic inhibitor derived from the Madagascar periwinkle plant (*Catharanthus roseus*). Vinca alkaloids act as anti-microtubule agents that block mitosis by arresting cells in the metaphase. Monochemotherapy with vincristine sulphate is effective, but treatment time may vary until complete clinical remission and is less toxic than other treatments (Choi *et al*., 2014 and Dour *et al.,* 2016). Vincristine sulphate is safe for most of the patients, but potential side effects can occur such as gastrointestinal alterations, myelosuppression and extravasation injury (Mason *et al*., 2014 and 2018).

Cisplatin, is a well-known chemotherapeutic drug. It has been used for treatment of numerous cancers including bladder, head and neck, lung, ovarian and testicular cancers. It is effective against several types of cancers, including carcinomas, germ cell tumours, lymphomas and sarcomas. Its mode of action has been linked to its ability to crosslink with the purine bases on the DNA, interfering with DNA repair mechanisms, causing DNA damage and subsequently inducing apoptosis in cancer cells (Cummings *et al*., 2000). However, because of drug resistance and numerous undesirable side effects such as severe kidney problems, allergic reactions, decrease immunity to infections, gastrointestinal disorders, haemorrhage and hearing loss especially in younger patients. Furthermore, combination therapies of cisplatin with other drugs have been highly considered to overcome drug resistance and reduce toxicity (Spugnini *et al*., 2019).

Hydrogel formulations of polymeric material from natural or synthetic sources combined with therapeutic agents have gained great attention in the recent years for treating various diseases (Haq *et al.,* 2017). New forms of treatments to attack cancer cells are required while simultaneously decreasing the side effects caused in healthy cells. There are several advantages from using encapsulated antineoplastic agents, including increased drug solubility, better bioavailability, high stability, controlled drug release, prolonged half-life, selective organs or tissue distribution and reduction of the total dose required. Hydrogels are three dimensional, hydrophilic polymeric networks which are capable of absorbing substantial amounts of water and biological fluids or molecules with ability to swell and dissolve in water. Hydrogels can be divided into chemical and physical gels depending on the nature of crosslinking. During apoptosis, the cell membrane loses its asymmetry and phosphatidyl-serine (PS) becomes exposed on the cell surface.

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How to cite this article: Kumar, A., Jadon, N.S., Zaidi, M.G.H., Kandpal, M., Sandhu, R.S. and Saini, R. (2024). Comparative Evaluation of Apoptotic Effects of Vincristine, Cisplatin and Their Hydrogel Scaffolds on HeLa Cell Line. Indian Journal of Animal Research. 58(4): 695-697. doi: 10.18805/IJAR.B-4412.

Submitted: 01-02-2021 **Accepted:** 26-03-2022 **Online:** 20-05-2022

Though there have been rapid improvements in technology and medical science, still the overall cancer incidence is on the rise. The scenario in veterinary field is more disapproving because of the exorbitant cost of therapy.

In the view of above facts, the present study on comparative evaluation of apoptotic effects of vincristine, cisplatin and their hydrogel scaffolds on HeLa cell line have been undertaken.

Application of vincristine sulphate and cisplatin scaffolds on HeLa Cells

HeLa cells obtained from the National Centre for Cell Sciences (NCCS), Pune were adapted to grow in DMEM (high glucose) supplemented with 10% FBS, penicillin 100 U/ml, streptomycin 100 µg/ml at 37°C with 5% CO_2. Subculture of HeLa cells in T25 flask and six well plate, for transfection with hydrogel scaffold of vincristine and cisplatin were done. Once the cells were ~ 80% confluent, trypsinized with 1 ml of 1X trypsin-EDTA, incubated at 37°C for 5 min and seeding cells were resuspended in DMEM-high glucose medium with 10% FBS, penicillin 100 U/ml, streptomycin 100 µg/ml and cells were seeded in T25 flask and six well plates at 37°C with 5% CO_2. Under these conditions, cells reached confluence in 3 days (Fig 1) and thereafter treated with the test compounds.

HeLa cells grown to 80% confluency in a T25 flask and six well plates were transfected with vincristine and cisplatin alone and their hydrogel scaffold. The mixture was incubated for 30 min at room temperature for complex formation. Meanwhile, cells were washed once with 1 ml of warm PBS and 1.5 ml of fresh cell growth medium without antibiotics (DMEM with 10% FBS) was added to each well. After that plate were incubated at 37°C and 5% CO_2 for 24 h. After 24 h, media were replaced with fresh growth medium and plate were incubated at 37°C and 5% CO_2 for expression.

Estimation of apoptic potential of drugs and scaffolds by DNA fragmentation (Ladder) assay

The DNA fragmentation assay were performed, wherein about 3×10^6 cells were treated with lysis buffer (1% NP-40 in 20 mM EDTA, 50 mM Tris-HCl, pH 7.5) and centrifuged at 10000× g for 10 min at 4°C. The supernatants were treated with 10% SDS for 2 h at 37°C followed by digestion with proteinase-k (220 µg/ml) at 50°C for 3 h. After the addition of half volume of 10 M ammonium acetate, the DNA were precipitated with 2.5 volume of absolute ethanol. The DNA pellet obtained were washed with 70% ethanol, air dried and reconstituted in 20 µl of Tris EDTA buffer containing RNase A (10 mM Tris, 1 mM EDTA and 100 µg/ml RNase) for 1 h at RT and then run on 1.8% agarose gel at 60 volts and observed for the specific ladder pattern.

Statistical analysis

Statistical analysis of the data was done with one way analysis of variance. Analysis of variance and C.D. test were done as per the methods described by the Snedecor and Cochran (1994).

Effects of drugs on HeLa cells

The apoptosis was assessed by DNA fragmentation test. Apoptosis can be visualized as a ladder pattern due to DNA cleavage by the activation of a nuclear endonuclease using standard agarose gel electrophoresis. Results showed the formation of the DNA ladder in gel electrophoresis by induction of apoptosis in HeLa cell line (Fig 2) treated with 20 µg/ml of vincristine alone, 20 µg/ml cisplatin alone and their scaffolds with, a typical ladder pattern of inter-nucleosomal fragmentation observed in cells after 24hrs treatment. Low molecular weight DNA from these cells was resolved in 1.8% agarose gels.

Genomic DNA from HeLa cells was isolated and subjected to electrophoresis in agarose gel (1.8%) and 1kb DNA ladder. DNA fragments were visualized under a UV trans-illuminator and compared with a standard marker. Lane C showed no fragmented DNA, but 20 µg/ml vincristine and 20 µg/ml cisplatin showed fragmented DNA in the form of ladder 1 and 3 after 24 h. Vincristine scaffolds and cisplatin scaffolds showed moderate DNA fragment in lane 2 and 4. The results suggest that scheduled vincristine

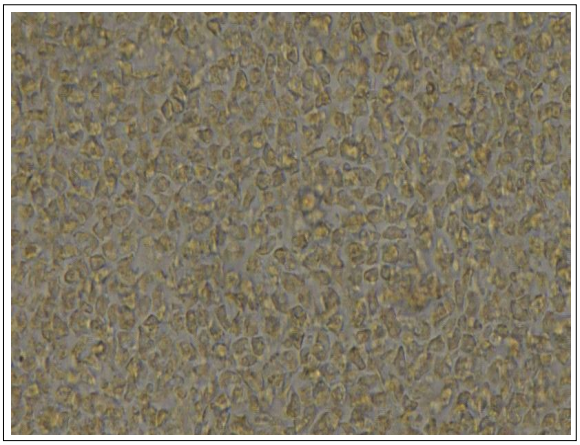

Fig 1: Microscopic image of HeLa cells confluent monolayer.

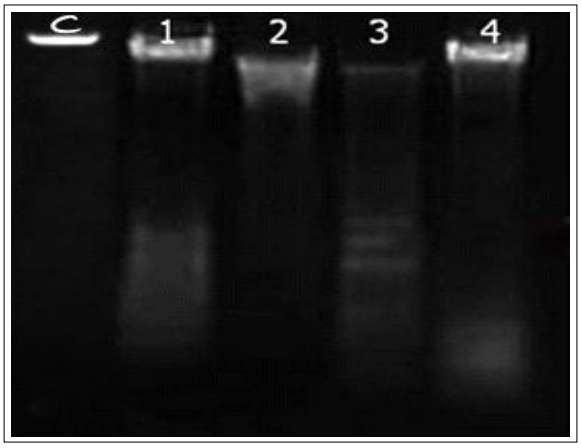

Fig 2: Photograph showing DNA Fragmentation Assay of HeLa cells.

sulfate applications stabilize the induction of tumor regression by inducing apoptosis and preventing cell proliferation.

Apoptosis can be visualized as a ladder pattern due to DNA cleavage by the activation of a nuclear endonuclease by standard agarose gel electrophoresis. Apoptosis is a physiological process of cell elimination and DNA fragmentation is one of the hallmarks of cell apoptosis. The results showed that the apoptosis proportion of cells was increased by treatment of vincristine and cisplatin alone and mild with their scaffolds in HeLa cells. Similar observations were also reported by Zhang and Guzalnur (2015) and Knudson, (2019).

CONCLUSION

Based on the observations made in this study, it was concluded that the formation of the DNA ladder in gel electrophoresis by induction of apoptosis in HeLa cell line treated with vincristine, cisplatin alone and their scaffolds showed best fragmentation in vincristine and cisplatin alone however, their scaffolds are moderately effective, when it is used in appropriate dose on HeLa cell line.

Conflict of interest

All authors declared that they have no conflicts of interest.

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