Journal of Animal Research: v.13 n.04, p. 623-627. August 2023

DOI: 10.30954/2277-940X.04.2023.19

Antiproliferative Effect of Methanolic and Aqueous Extract of *Duranta erecta* in C127I Cell Line

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Received: 29 May, 2023 **Revised:** 10 July, 2023 **Accepted:** 20 July, 2023

ABSTRACT

The Phytochemicals and their derivatives found in plants are most promising alternatives to improve treatment regimens in cancer patients with less adverse effects. Methanolic and aqueous extracts of *D. erecta* were assessed for their cytotoxicity in C127I cell line by 3-(4,5-dimethyl thazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay at concentrations of 320, 160, 80, 40, 20 and 10 µg/mL and the half maximal inhibitory concentration (IC50) was calculated using Graph Pad Prism 5.0. Doxorubicin was used as positive control. Dose dependent reduction in cell viability was noticed when the cells were subjected to different concentrations of the extracts. The IC_{50} of the aqueous and methanolic extract of *D. erecta* were 41.58 and 44.66 µg/mL respectively. The cells were seeded in 6 well plates at a concentration of 1×10^5 cells/mL and were treated for 24 hours with methanolic and aqueous extract of *D. erecta* at concentration of IC_{50}. The cells were trypsinised and subjected to Acridine orange – Ethidium bromide staining (AOEB) staining detected nuclei of normal cells stained by AO penetration which dyes green via attaching to DNA, EB, on the other hand, dyes the nuclei of late apoptotic and necrotic cells red and the result shows that *D. erecta* and doxorubicin induced apoptosis in a dose dependent manner. In conclusion, this study established that the methanolic and aqueous extract of *D. erecta* induces apoptosis in cancer cells in a dose dependent manner could be developed as a lead molecule for cancer management after conducting clinical trials *in vivo* and human subjects.

HIGHLIGHTS

- The methanolic and aqueous extract of *D.erecta* induces apoptosis in cancer cells in a dose dependent manner
- The IC_{50} of the aqueous and methanolic extract of *D. erecta* were 41.58 and 44.66 µg/mL respectively.

Keywords: C127I, *D. erecta*

One of the most important crippling disease that affect both human beings and animals is cancer which accounts for more deaths worldwide. Malignancies usually get carried over to different organs from organ of origin where they cause metastasis and cause more damage than the affliction of primary organ. Breast cancer accounts for more than one in ten new cancer diagnosed each year and is most common cause of mortality in women. It is the second most common cause of death from cancer among women in the world (Growder *et al*., 2020). The treatments for early-stage and locally advanced breast cancer include surgery, radiation therapy, chemotherapy, hormonal and targeted therapies, immunotherapy etc.

Medicinal plants has been used widely for the treatment of ailments globally and natural products has been corner stone for the development of many drugs that are cytotoxic that can augment the current existing therapies

How to cite this article: Thomas, S., Roshni, S.S., Geevargheese, S., Mohanan, R., Sujith, S., Begum, N., Nisha, A.R., Koorse, K.G. and Surya, S. (2023). Antiproliferative Effect of Methanolic and Aqueous Extract of *Duranta erecta* in C127I Cell Line. *J. Anim. Res.,* **13**(04): 623-627.

Source of Support: None; **Conflict of Interest:** None

against cancer (Green and Rahman, 2015). Various phytomolecules that have been developed as anticancer drugs from plants include epipodophyllotoxin (Unnati *et al*., 2013), antioxidants like resveratrol, gallacatechins (Amos and Lowe, 1999), vincristine (Jordan and Wilson 2004), various flavonoids and steroids (Growder *et al*., 2020). Duranta is native plant of Asia, Africa, South and Central America, belongs to the Verbenaceae family. *Duranta erecta* commonly known as "golden dew drop," is an upright scrambling shrub which is grown for its ornamental purpose, has ethnomedicinal applications as larvicidal (Serena *et al.,* 2010), vermifuge, febrifuge, diuretic, anti-parasitic (Krishnaprasad *et al.,* 2018), and anti-malarial properties. *D. erecta* is also a major source of phenylethanoid glycoside known as acteoside-a drug used in clinical trials for IgA nephropathy patients (Madhumita and Shanker, 2022). The phytoconstituents isolated from *D. erecta* include coumarinolignoids, (E)-pmethoxycinnamic acid, (E)-cinnamic acid, lamiide, β-sitosterol, raffinose, naringenin, sucrose and raffinose (Iqbal *et al.,* 2004). The present study was undertaken to identify the various phytochemical constituents in the methanolic and aqueous extracts of *D. erecta* and to evaluate the cytotoxicity of the extracts in C127I cell line.

MATERIALS AND METHODS

Plant material

The fruits of *Duranta erecta* was collected locally, dried under shade and extracted using methanol in a soxhlet extraction apparatus. The dried fruits were extracted using water as a decoction, both extracts were further concentrated in rotary vacuum evaporator at temperature 40° C under reduced pressure. The qualitative phytochemical analysis of the extracts was performed to test the presence of bioactive ingredients according to Harborne (1998).

Cell lines

C127I, mouse mammary tumor cell line obtained from the National Centre for Cell Sciences in Pune, were used in study. The cells were cultured in 25 cm^2 tissue culture flaks with the supplementation of Dulbecco's Modified Eagle Medium (DMEM) with 10 per cent foetal bovine serum, one per cent Gentamicin (50 mg/mL) and incubated for 24 hrs at 37°C in CO_2 incubator during the course of the experiment.

Cytotoxicity studies: 3-(4,5- dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay

In-vitro cytotoxic potential of the methanolic and aqueous extract of *D. erecta* was assessed in C127I, using MTT reduction assay (Dhanusha *et al.,* 2021). Microtiter plate (96 wells) plates were seeded with 5×10^4 cells/mL and incubated for 24 hrs to achieve cell attachment. After incubation the cells were exposed to extracts diluted to concentrations 320, 160, 80, 40, 20 and 10 µg/mL and incubated for 24 hrs. Doxorubicin was used as positive control. After 24 hours of incubation with the extract, MTT at a concentration 5mg/mL was added to each well at 10 µL, incubated for 4 hours with 100 µL serum free media. The reaction was stopped by adding 100 µL of DMSO to all the wells to dissolve to formazan crystals formed. The absorbance was measured using microplate reader at a wavelength of 594 nm. Per cent cell viability, Per cent cell inhibition were evaluated and IC_{50} values of extract was calculated using Graph Pad Prism 5.0.

Acridine orange / Ethidium bromide (AO/EB) staining

The cells were seeded in 6 well plates at a concentration of 1×10^5 cells/mL and allowed to incubate for 24 hours to achieve cell attachment. After incubation, the cells were treated for 24 hours with the extract of *D. erecta* at IC_{50} concentration. The acridine orange / ethidium bromide (AO/EB) staining procedure was followed to differentiate the live, apoptotic and necrotic cells. Twenty-five microlitres of the treated or untreated cells were stained with five microlitres of acridine orange (10 µg/mL) and ethidium bromide (10 µg/mL) and analysed under Trinocular Research fluorescence microscope, DM 2000 LED, Leica with blue excitation (488 nm) and emission (550 nm) filters at 10X magnification (Sandesh *et al*., 2022).

RESULTS

The phytochemical analysis revealed the presence of steroids, alkaloids, tannins, flavonoids, glycosides, cardiac

glycosides, phenolic compounds, diterpenes, triterpenes and saponins. The results were summarised in table 1.

Table 1: Phytochemical analysis of methanolic extract and methanolic and aqueous extract of fruits of *Duranta erecta*

Sl. No.	Phyto-constituents	Methanolic extract of fruits of *Duranta erecta*	Aqueous extract of fruits of *Duranta erecta*
1	Steroids	+	+
2	Alkaloids	+	-
3	Tannins	+	+
4	Flavonoids	+	+
5	Glycosides	+	+
6	Cardiac Glycosides	+	+
7	Phenolic compounds	+	+
8	Diterpenes	+	+
9	Triterpenes	+	+
10	Saponins	+	+

Cytotoxic evaluation of methanol extract of *Duranta erecta* in C127I cell line

Dose dependent reduction in cell viability was noticed when the cells were subjected to different concentrations of the extract (table 2) and IC_{50} value of methanolic and aqueous extracts of *D. erecta* was found to be 44.66 µg/mL and 41.58 µg/mL respectively.

Table 2: The per cent cell viability of C127I cells after 24 hours treatment with methanolic and aqueous extracts of *D. erecta*

Concentration of the extract (µg/mL)	Methanolic extract	Aqueous extract
320	-6.2 ± 7.4	7.6 ± 1.6
160	22.2 ± 4.6	12.0 ± 1.8
80	46.0 ± 4.4	22.8 ± 2.0
40	69.9 ± 4.8	32.0 ± 5.0
20	67.3 ± 4.5	65.5 ± 0.1
10	88.1 ± 7.2	136.7 ± 31.3
5	96.0 ± 5.7	106.2 ± 7.4

Values are expressed as Mean±SE, with n=3 replicate.

Maximum inhibition was seen when cells were treated with 320 µg/mL of methanolic extract of *D. erecta* with value 96.0 ± 5.7 per cent and for aqueous extract, maximum viability was seen when cells were treated with 10 µg/mL with value 136.7 ± 31.3 per cent. The per cent cell inhibition C127I cells after 24 hours treatment with methanolic and aqueous extracts of *D. erecta* is depicted in table 3. The results were comparable to that of doxorubicin.

Table 3: The per cent cell inhibition C127I cells after 24 hours treatment with methanolic and aqueous extracts of *D. erecta*

Concentration (µg/mL)	Methanolic extract	Aqueous extract
320	106.2 ± 7.4	92.4 ± 1.6
160	77.8 ± 4.6	88.0 ± 1.8
80	54.0 ± 4.4	77.2 ± 2.0
40	30.1 ± 4.8	68.0 ± 5.0
20	32.7 ± 4.5	34.5 ± 0.00
10	11.9 ± 7.2	-36.7 ± 31.3
5	4.0 ± 5.7	-6.2 ± 1.0
IC_{50}(µg/mL)	44.66	41.58

Values are expressed as Mean±SE, with n=3 replicate.

Acridine Orange/ Ethidium Bromide staining

After treatment with extract, acridine orange / ethidium bromide (AO/EB) staining procedure was followed and the live, apoptotic and necrotic cells were differentiated. The pictures of cells exposed to different treatments are illustrated in Fig. 1. In the early apoptotic cells, the nuclei were crescent shaped or granular, which where stained yellow or green.

DISCUSSION

Apoptosis or programmed cell death is one of the major mechanisms by which body controls the unwanted proliferation of cells through various signalling pathways (Reed, 2000). When the cell turn over is counter balanced by cell proliferation, there will be formation of tumor which when becomes uncontrolled by various check mechanism becomes cancer (Reed, 1999). A variety of drugs are being synthesised which can control various stages of apoptosis, forcing the tumor cells to undergo apoptosis and thus control cell proliferation. Among

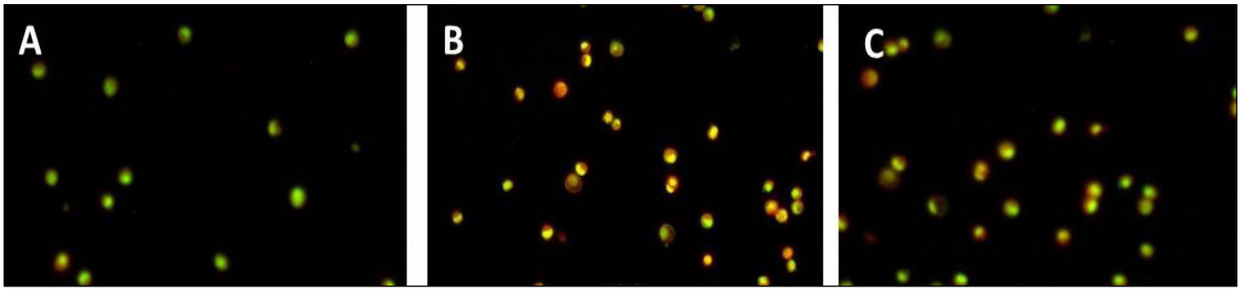

Fig. 1: Pictures of AO/EB staining: **(A)** normal cells, **(B)** methanolic extract of *D. erecta* at IC_{50}, **(C)** aqueous extract of *D. erecta* at IC_{50}

these proapoptotic anticancer agents, plant products form a major bulk which acts through various mechanisms possessing limited side effects (Wu *et al*., 2002). In the present study the *in-vitro* cytotoxic potential of the methanolic as well as aqueous extract of *D. erecta* were assessed in C127I cell line using MTT reduction assay. Cell viability assays are used to detect the antiproliferative potential of various drugs *invitro*. In MTT assay, the active cells aid in the conversion of MTT dye to purple formazan crystals which produces a proportionate change in the optical density at 570 nm (Abdel *et al*., 2012; John *et al*., 2020). In the present study, there was a dose dependent reduction in cell viability was noticed when the cells were subjected to different concentrations of the extract and the positive control doxorubicin. The IC_{50} of the aqueous and methanolic extract were 41.58 and 44.66 µg/mL respectively. According to the NCI standards, they possess moderate cytotoxicity.

Apoptosis is a physiological process of cell elimination and the ability to induce apoptosis is an important property of the anticancer agents. Acridine orange/ethidium bromide dual staining is a used to detect apoptosis based on the differential uptake of the two fluorescent DNA binding dyes to determine the live, early, and late apoptotic cells (Bimitha *et al*., 2022). The nuclei of normal cells stained by AO penetration which green via attaching to DNA, EB, on the other hand, dyes the nuclei of late apoptotic and necrotic cells red (Sandesh *et al.,* 2022) and the result shows that *D. erecta* and doxorubicin induced apoptosis in a dose dependent manner. The extracts showed the presence of steroids, alkaloids, tannins, flavonoids, glycosides, cardiac glycosides, phenolic compounds, diterpenes, triterpenes and saponins and is similar to other findings (John *et al*., 2020). Thus, every identified phytochemical of the methanolic and aqueous extract of *D. erecta* has its bioactivity for therapeutic values which can be used in the therapeutic formulations in a prudent and worthwhile manner.

CONCLUSION

In conclusion, this study established that the methanolic and aqueous extract of *D. erecta* induces apoptosis in cancer cells in a dose dependent manner could be developed as a lead molecule for cancer management after conducting clinical trials in vivo and human subjects.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance provided by Kerala veterinary and Animal Sciences University for the conduct of this research work.

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