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Original Article

CYTOTOXICITY AND APOPTOTIC INDUCIBILITY OF HYDRO-ETHANOLIC EXTRACT OF AERIAL PARTS OF *POTHOS SCANDENS* L. AGAINST MCF-7 BREAST CANCER CELL LINE

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ABSTRACT

Objective: To investigate the ability of 50% hydro-ethanolic extract of *Pothos scandens* (*P. scandens*) to induce cytotoxicity and apoptosis in MCF-7 cell lines.

Methods: Aerial parts of *P. scandens* were extracted with 50% ethanol. The crude extract obtained was evaluated for its ability to induce cytotoxicity against MCF-7 (breast cancer) and L929 (normal fibroblast) cell lines by MTT (3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay. The ability to induce apoptosis was determined by acridine orange (AO) and ethidium bromide (EB) double staining method. The above activity was confirmed using the comet assay.

Results: The study revealed that the plant extract showed significant cytotoxic activity against MCF-7 cell lines with an IC_{50} of 90.18±5.20 µg/ml. Further it was also found that the cell death of MCF-7 treated with crude extract was due to the induction of apoptosis, which was ascertained by comet assay.

Conclusion: The study provides scientific evidence for the anticancer activity of extracts of *P. scandens* paving a way to further research of using this plant in the development of the novel anticancer drug.

Keywords: *P. scandens*, MCF-7, L929, MTT assay, Apoptosis, Comet assay

INTRODUCTION

Cancer is the third leading cause of death worldwide, only preceded by cardiovascular disease, infectious and parasitic disease [1]. Despite a large number of studies, we are still uncertain as to the cause of the most type of human cancer [2]. Breast cancer is the most common form of cancer and the leading cause of cancer mortality among women worldwide. It is estimated that one out of eight women develop breast cancer in their lifetime [3, 4]. It is a serious clinical problem that possesses significant social and economic challenges to the health care system [5].

Though there are many therapeutic strategies including chemotherapy to treat cancer, high systemic toxicity and drug resistance limit the successful outcomes in most cases. Moreover, cancer treatment is usually accompanied by diverse side effects to different body organs [6]. Multidisciplinary scientific investigations are making best efforts to combat this disease, but the sure-shot, perfect cure is yet to be brought into the world of medicine [7, 8] and hence there is a worldwide trend to go back to natural resources (medicinal plants) which are therapeutically effective, culturally acceptable and economically affordable [9]. As part of this effort, many natural products have been tested against various types of cancer cell lines [10] and still there has been a long-standing interest in the identification of medicinal plants and derived natural products for developing cancer therapeutics [11].

Pothos scandens (*P. scandens)* is a medicinal aroid, which belongs to the family Araceae. Many medicinal properties of this plant have been reported. The bruised root of the plant is applied to promote healing of abscesses, after being fried in oil. It has also been reported that the whole plant is used against various health problems and disorders such as diarrhea, smallpox, etc. According to the ethnobotanical data collected during the field surveys made on several visits between 2004 and 2006 to three Akha communities in Chiang Rai in northern Thailand, it was found that the traditional healers use whole aerial parts of *P. scandens* to treat cancer [12-14]. But so far the anticancer property of this plant has not been studied. Hence, the present study aims to investigate the therapeutic properties of *P. scandens* for its potential anticancer activity by evaluating their cytotoxicity and apoptotic ability against MCF-7 breast cancer cell lines.

MCF-7 breast cancer cell lines and L929 (Fibroblast cells) cell lines were obtained from National Center for Cell Sciences (NCCS), Pune and was maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37 °C in 5 % CO_2 in a humidified atmosphere in a CO_2 incubator (NBS, EPPENDORF, Germany). All other chemicals are of the standard analytical grade.

P. scandens were collected from in and around Palai, Kottayam, Kerala. They were identified and certified by the Taxonomist, Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India (Plant identification no.-BSI/SRC/5/23/2013-14/Tech/685). The aerial parts of *P. scandens* were shade dried and ground to coarse powder. The coarse powder was extracted using 50% ethanol using soxhlet apparatus for 48 h. The extracts were condensed to dryness using rotary evaporator.

Cytotoxic effects of *P. scandens* against MCF-7 and L929 cells were assessed by MTT (3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay [15]. The ability of the extract to induce apoptosis of MCF-7 was determined by acridine orange (AO) and ethidium bromide (EB) double staining method [16]. The above activity was confirmed using the comet assay (alkaline version) as described by Singh *et al.* [17]. All experiments were performed in triplicate (n=3) and the results were expressed as mean±SD. Statistical analysis was carried out using SPSS 16.0.

MTT assay is based on the conversion of yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells. The

amount of formazan produced is proportional to the number of viable cells. The study was carried out to evaluate the cytotoxic effect of 50% ethanolic extract of *P. scandens* on MCF-7 and L929. It was found from the study that the extract showed a significant cytotoxic effect against MCF-7 cell lines in a dose-dependent manner. The extract showed a little cytotoxic effect against L929 cell lines used. The viability of the MCF-7 cell lines which was 78.35±2.14 % at a concentration of 6.25 µg/ml was decreased to 50.54±1.67 % at a concentration of 100 µg/ml. The viability of L929 cell lines were 96.26±0.78 % and 76.98±1.72 % at 6.25 µg/ml and 100 µg/ml concentrations respectively (fig. 1). The IC_{50} of 50% ethanolic extract of *P. scandens* for MCF-7 cell line was found to be 90.18±5.20 µg/ml. MTT assay is commonly used in cell biology for the study of growth factor, cytokines and cytotoxicity of chemotherapeutic agents as it offers a quantitative and simple method for evaluating cell population's response to external factors. In the present study, it was found that the plant extract decreased the viability of MCF-7 cell lines in a dose-dependent manner at the same time being less effective against L929-normal fibroblast cell lines. It can be explained that the reduction in the number of cells by particular agents (cytotoxicity) may be due to cell and/or cell proliferation [18].

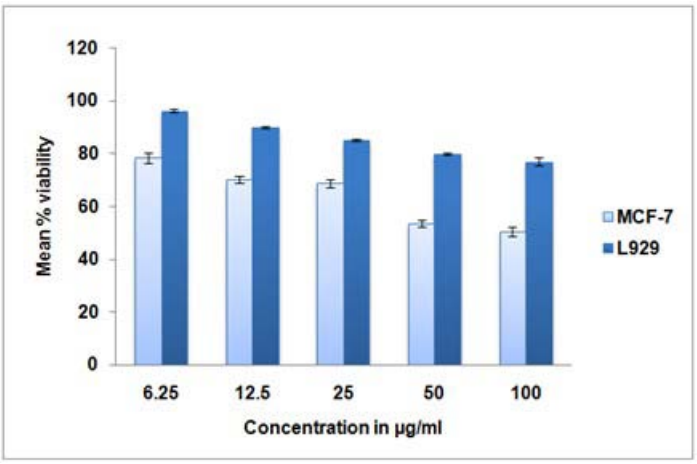

Fig. 1: Cytotoxic effect of 50% ethanolic extract of *P. scandens* on MCF-7 and L929 cell lines after 24 h treatment. Each data point represents the mean from three independent experiments (mean±SD)

Most anti-cancer drugs induce apoptosis, as a primary mechanism for inhibition of cell proliferation. In order to determine whether the decrease in cell viability was due to apoptosis, a morphological change at cellular and nuclear levels was assessed with cells treated with 50% ethanolic extract of *P. scandens*. AO selectively stains the living cells as green whereas EB stains dead cell DNA as red. Microscopic analysis of cellular and nuclear morphologies suggests the presence of both apoptotic and necrotic cells. The untreated control MCF-7 cell lines were characterized by bright green nucleus with uniform intensity and the absence of EB uptake. Nuclear condensation and fragmentation, hallmark features of apoptosis were present in the plant extract treated cells in a dose dependent manner (fig. 2).

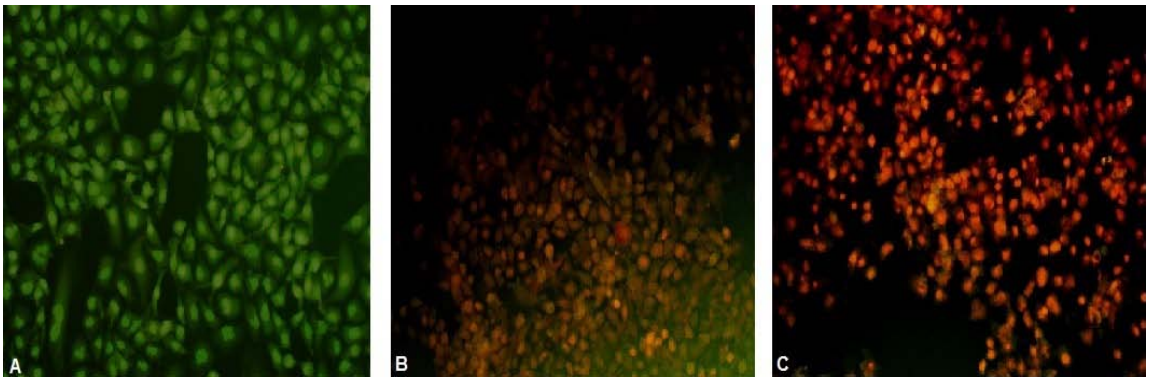

Fig. 2: Analysis of apoptosis by AO and EB double staining: Nuclear uptake of dyes depends on membrane integrity and based on that cells are divided into three categories: (i) green fluorescent nuclei show intact cells, (ii) orange coloured nuclei show early apoptotic cells and (iii) dark red colored cells show fragmented nuclei evident for late apoptotic or necrotic cells. (A) Untreated MCF-7 cells (B & C) MCF-7 cells treated with 50µg/ml and 100µg/ml of 50% ethanolic extract of *P. scandens* respectively. Data are representative of three independent experiments

Apoptosis, identified as one of the most fundamental biological processes in eukaryotes in which individual cells die by activating intrinsic "suicide" mechanisms, has been suggested to play a key role in damage to cells [19]. The results of the study indicate that the plant extract resulted in apoptosis of MCF-7 cell lines which again support the cytotoxic activity of the plant. Our observations of altered nuclear morphology after treatment with the *P. scandens* extract (fig. 2: B & C) are consistent with previous reports [20, 21] of cells undergoing apoptosis. Comet assay is a sensitive and reliable technique for evaluating the presence and level of DNA strand breaks [22].

In the present study, MCF-7 cells treated with extract at IC_{50} showed fluorescence intensity that increased in the comet tail, revealing that the fragments of DNA were excluded from their nuclei, while the control cells displayed only fluorescent nuclei without comet tails (fig. 3).

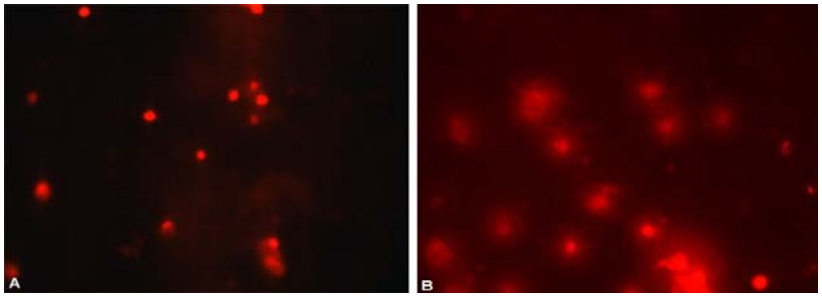

Fig. 3: Representative slides of comet profiles for MCF-7 cells visualized with fluorescence microscopy. (A) Control untreated MCF-7cellS (B) MCF-7 cells treated with 90.18 µg/ml (IC_{50}), 50% ethanolic extract of *P. scandens*. Data are representative of three independent experiments

As cellular DNA damage becomes serious, more DNA fragments migrate into the comet tail region and are quantified by the intensity of fluorescence according to comet parameters including tail length, tail intensity and Olive tail moment, representing the levels of DNA damage [23]. Our studies indicate that fluorescence intensity increased in the comet tail, revealing that the fragments of DNA were excluded from their nuclei, while control cells displayed only fluorescent nuclei without comet tails, after exposure to IC_{50} concentration.

The ability of *P.* scadens to induce cytotoxicity may be attributed to the presence of phytochemicals like phenolic and flavonoid compounds present in the plant [24]. The presence of flavonoid and phenolic compounds in the plant has been reported in previous works [25, 26].

To the best of our knowledge, this is the first publication about the cytotoxic and apoptotic effect of *P. scandens* extract. From the study, it is concluded that the plant is having significant anticancer activities against MCF-7 breast cancer cell lines. Studies are on the way to evaluate the efficacy of *P. scandens* against breast cancer in animal models.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests

REFERENCES

1. Mathers CD, Boschi-Pinto C, Lopez AD, Murray CJL. Cancer incidence, mortality and survival by site for 14 regions of the world. World Health Organization; 2001. p. 3.
2. Gerald Karp. Cell and molecular biology. 3rd Edition. John Wiley and Sons, Inc; 2003. p. 671-702.
3. Smigal C, Jemal A, Ward E, Cokkinides V, Smith R, Howe H, *et al.* Trends in breast cancer by race and ethnicity: Update. CA Cancer J Clin 2006;56:106-30.
4. Russo J, Yang X, Hu Y. Biological and molecular basis of human breast cancer. Front Biosci 1998;3:944-60.
5. Guo J, Wang MH. Extract of *Ulmus davidiana* planch barks induced apoptosis in human hepatoma cell line HEPG2. Exp Clin Sci J 2009;8:130-7.
6. Saalu LC, Ajayi GO, Adeneye AA, Imosemi IO, Osinubi AA. Ethanol seed extract of grapefruit (*Citrus paradisi*) as an effective attenuator of doxorubicin-induced oxidative stress in the rat heart. Int Cancer Res 2009;5:44-52.
7. English DR, Armstrong BK, Kricker A, Fleming C. Sunlight and cancer. Cancer Causes Control 1997;8:271-83.
8. Kuper H, Boffetta P, Adami HO. Tobacco use and cancer causation: association by tumour type. J Intern Med 2002;252:206-24.
9. Jeyachandran RA, Mahesh, Cindrella L. DEN-induced cancer and its alleviation by *Anisomeles malabarica* L R. Br. ethanolic leaf extract in male albino mice. Int J Cancer Res 2007;3:174-9.
10. Capua CJ, Hopson NP, Stewart CMM, Johnston GR, Neill KLO, Schaalje GB, *et al*. Cytotoxicity of *Atriplex confertifolia*. J Toxicol 2010;1155:1-7.
11. George S, Bhalerao SV, Lidstone EA, Ahmad IS, Abbasi A, Cunningham BT, *et al*. Cytotoxicity screening of Bangladeshi medicinal plant extracts on pancreatic cancer cells. BMC Complementary Altern Med 2010;10:52.
12. Sangwoo L, Chunjie X, Shengji P. Ethnobotanical survey of medicinal plants at periodic markets of honghe prefecture in Yunnan Province, SW China. J Ethnopharmacol 2008; 117:362-77.
13. Baharuddin S, Mansor M. Medicinal aroids conservation: a case study of a floral garden. School of Biological Scie Ces, Universiti Sains Malayasia. Proceedings of the 4th IMT-GT UNINET Conference; 2002. p. 216-9.
14. Angkhana I, Shengji P, Balslev H, Wong PW, Trisonthi C. A comparative study on medicinal plants used in Akha's traditional medicine in China and Thailand, cultural coherence or ecological divergence? J Ethnopharmacol 2008;116:508-17.
15. Enos Tangke Arung, Britanto Dani Wicaksono, Yohana Ayupriyanti Handoko, Irawan Wijaya Kusuma, Dina Yulia, Ferry Sandra. Anti-cancer properties of diethyl ether extract of wood from Sukun (*Antocarpus altilis*) in human breast cancer (T47D) cells). Trop J Pharm Res 2009;8:217-324.
16. Zhang JH, YU J, Li WX, Cheng CP. Evaluation of Mn^{2+} stimulated, and Zn^{2+} inhibited apoptosis in rat corpus luteal cells by flow cytometry and fluorochromes staining. Chin J Physiol 1998;41:121-6.
17. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184–91.
18. Smit HF, Woerdenbag HJ, Singh RH, Meulenbeld GH, Labadie RP, Zwaving JH. Ayurvedic herbal drugs with possible cytostatic activity. J Ethnopharmacol 1995;47:75-84.
19. R and D Systems: Cytokine Bulletin: Cytochine-Related Mechanisms of Apoptosis, R and D Systems, Minneapolis, MN; 1997.
20. Kerr JFR, Winterford CM, Harmon BV. Apoptosis. It's significance in cancer and cancer therapy. Cancer 1994;73:2013-26.
21. Sadegh Fattahi, Ali Motevalizadeh Ardekani, Ebrahim Zabihi, Zeinab Abedian, Amrollah Mostafazadeh, Roghayeh Pourbagher, *et al*. Antioxidant and apoptotic effects of an aqueous extract of *Urtica dioica* on the MCF-7 human breast cancer cell line. Asian Pacific J Cancer Prevention 2013;14:5317-23.
22. Dhawan A, Bajpayee M, Parmar D. Comet assay a reliable tool for the assessment of DNA damage in different models. Cell Biol Toxicol 2009;25:5-32.
23. Cok I, Ulutaş OK, Okuşluk O, Durmaz E, Demir N. Evaluation of DNA damage in common carp (*Cyprinus carpio L*) by comet assay for determination of possible pollution in lake mogan (Ankara). Sci World J 2011;11:1455-61.
24. Havsteen B. Commentary: flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol 1983;32:1141-8.
25. Lalitharani, Mohan VR, Maruthupandian A. Pharmacognostical and phytochemical studies on *Pothos scandens L.* Int J Phytomed 2010;2:277-83.
26. Saurabh Gupta, Duraiswamy B, Satishkumar MN. Peritoneal mast cell stabilization potential of *Pothos scandens L*. Indian J Pharmacol 2013;45:83-6.