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Cytotoxic Activity of *Amorphophallus paeoniifolius* Tuber *Extracts In vitro*

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Abstract: *Amorphophallus paeoniifolius,* belongs to the family Araceae, widely distributed in tropical and subtropical regions and are extensively used in South India for various diseases. The present investigation evaluates the cytotoxic property of the different solvent extracts of *A. paeoniifolius* tuber using *Allium cepa* L. root tip cells and HEp-2 cell line as two model *in vitro* systems. Of the seven different extracts of *Amorphophallus* tuber were tested, the mitotic index and cytolytic index were found to be high in petroleum ether and ethanol fractions when compared with other solvent extracts. The magnitude of cytotoxicity was predominant in petroleum ether extract and ethanolic extract and displayed a dose dependent antiproliferative activity on HEp2 cells. Present study thus confirms the cytotoxic property of *A. paeoniifolius* and also demonstrated the role of *A. paeoniifolius* used in the traditional medicine.

Key words: *Amorphophallus paeoniifolius* · medicinal plants · antiproliferative activity · *Allium cepa* L. root tip cells · HEp-2 cell line

INTRODUCTION

In recent years the popularity of complementary medicine has increased. Over 50% of all modern clinical drugs are natural product origin and they play an important role in drug development programs of the pharmaceutical industry [1]. Epidemiological evidence suggests that dietary factors play an important role in human health and in the treatment of certain chronic diseases including cancer [2, 3]. Some dietary sources contain antitumor compounds [4] and such compounds are candidates for chemo preventive agents against cancer development [5]. The anticancer property of nutrients derived from plants as well as nonnutritive plant derived constituents has been proved in different *in vitro* and *in vivo* models [6], which had led to an increased emphasis on cancer prevention strategies in which these dietary factors are utilized [7]. Dietary measures and traditional plant therapies as prescribed by ayurvedic and other indigenous systems of medicine are used commonly in India [8].

*Amorphophallus paeoniifolius, (*Araceae) is a commonly available tuber in South India, widely used in folk medicine for acute rheumatism, tumors, lung swelling, asthma, vomiting and abdominal pain. It is also a major ingredient of several Indian herbal prescriptions. So far, no attempts have been made to evaluate the medicinal properties of *A. paeoniifolius*. Hence the present study was performed to investigate the cytotoxic effects of different solvent extracts of *A. paeoniifolius* using *Allium cepa* L. root tip cells as well as HEp-2 cell line (a human larynx epithelial carcinoma cell line).

MATERIALS AND METHODS

Plant material: *Amorphophallus* tuber was collected from Coimbatore, Tamilnadu. The tuber was identified with the Herbarium of Botanical Survey of India, Southern Circle, Coimbatore, as *Amorphophallus paeoniifolius* and was deposited in the Department of Biotechnology, Bharathiar University.

Tuber extract preparation: The tuber of the plant was dried under shade and made to a fine powder (particle size ~0.25mm) using a laboratory mill and was extracted subsequently with a series of organic solvent with increasing polarity by using soxhlet extractor. The order of extraction was petroleum ether, benzene, chloroform, ethyl-acetate, acetone, ethanol and methanol. The isolated fractions were weighed and yield was calculated. Further, the extracted fractions were analyzed for its antiproliferative properties.

Plant cytotoxicity analysis: The extracts (1% w/v, in DMSO) of *A. paeoniifolius* tuber were subjected to plant cytotoxicity analysis. For this study *Allium cepa* L. root

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tip cells were used. Onions were placed with aerated water at room temperature to root for 24 h. DMSO was used as control. The control group was considered as time zero (0-h) until the first root sample was obtained. This root sample was then placed for 24h in extracts of the *A. paeoniifolius* tuber. After this time period a few root tips were removed and the bulbs were returned to water, for further 24h, to observe if there was recovery from possible damage. The treated roots were fixed and stained by acetocarmine and mounted on permanent slides. The slides were analyzed under microscope with 40X objective lens. Cells were examined for morphological and structural alterations and the mitotic index and cytolytic index were determined [9].

Animal cell cytotoxic assay: Cytotoxic assay was performed as described by Tengchaisri *et al.* [10] using HEp-2 cell line. Briefly, HEp-2 cells suspended in Minimal essential medium (MEM) containing 10% FBS were seeded at 1×10^4 cells (100 µl) per well in 96-well plate and incubated in humidified atmosphere with 95% and 5% CO_2 at 37°C. After 24 h, additional medium (100µl) containing the test compound (different concentrations) dissolved in 0.2% DMSO was added and further incubated for 24 h. The viability in cultured cells was determined by trypan blue exclusion assay. Cells were harvested using 0.025% trypsin, incubated with 4% trypan blue solution and were counted using a hemocytometer under light microscope. Cells failing to exclude the dye were considered non viable and the number of nonviable cell was expressed as a percentage of the total cells.

RESULTS

In the present study, *A. paeoniifolius* tuber was extracted with organic solvent. The extracts were analyzed for its antiproliferative properties using *Allium cepa* L. root tip cells and HEp-2 cells.

Tuber of *A. paeoniifolius* was extracted subsequently with petroleum ether, benzene, chloroform, ethyl acetate, acetone, ethanol and methanol. The percentage yield of this extraction ranged between 1 to 7% (Fig. 1). The highest yield of 6% and 6.7% were observed in case of petroleum ether extraction and ethanol extraction.

All the seven extracts of *Amorphophallus* tuber were subjected to plant cytotoxicity analysis using *Allium cepa* L. root tip cells. Only petroleum ether and ethanol showed low mitotic index of 0.34% and whereas the cytolytic index were found to be 75% for petroleum ether and 80% for ethanol extract. The mitotic index and cytolytic index were found to be low in chloroform, acetone and methanol. The petroleum ether and ethanol were found to be highly antiproliferative property (Table 1).

Table 1: Plant cytotoxicity analysis in *Allium Cepa* L. root tip of different extracts of *A. paeoniifolius*

Treatments	Total number of cells	Mitotic index (%)	Cytolytic index (%)
Control (DMSO)	148	11.90	1.2
Petroleum ether	100	0.34	75.0
Benzene	85	3.50	15.0
Chloroform	82	1.40	9.0
Acetone.	150	5.00	4.8
Ethyl acetate	148	2.40	5.9
Ethanol	145	0.34	80.0
Methanol	105	6.22	6.0

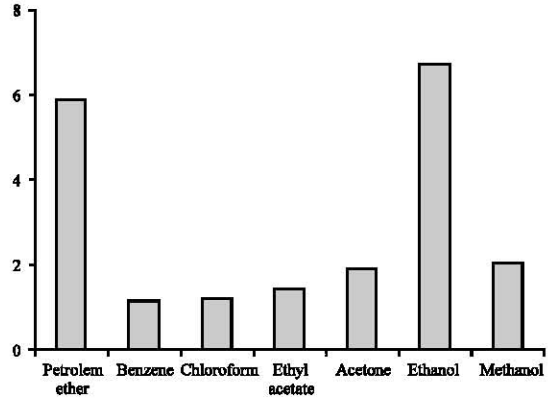

Fig. 1: Percentage yield of constituents in different extraction of *A. paeoniifolius*

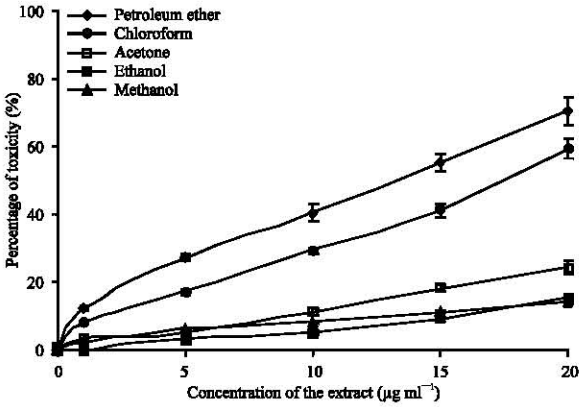

Fig. 2: Cytotoxicity of different extracts of *A. paeoniifolius* on HEp2 Cell lin

Of the seven different extracts of *Amorphophallus* tuber were tested, only ethanol and petroleum ether extract displayed a dose dependent antiproliferative activity on HEp_2 cells (Fig. 2). In the present study, HEp_2 epidermoid cell line was used which is the best model to

study the cytotoxicity assay. Untreated Hep-2 cells appeared as elongated shape, attached smoothly on the culture surface and some of the cells grouped together to form colonies. Following treatments with extract for 24 hrs, the cells changed to round shape and lost cell contacts. In particular, the cells lost their surface morphology and died at a concentration of 60 and 70%. Further the viability assay using trypan blue dye showed the maximum number of death percentage in petroleum ether extract when compared to ethanol extract which showed the secondary level of death of cells. Whereas the magnitude of cytotoxicity was predominant in petroleum ether extract and ethanolic extract when compared to other extracts. Since the cytotoxicity was not determined in ethyl acetate and benzene extract (Data not given), however very less antiproliferative activity was observed in chloroform and acetone extract.

DISCUSSION

Plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use [11]. Agents capable of inhibiting cell proliferation, inducing apoptosis or modulating signal transduction are currently used for the treatment of cancer [12]. The use of multiple chemopreventive agents or agents with multiple targets on cancer cells are considered to be more effective in cancer treatment [13].

An assessment of their cytotoxic and mutagenic potential is necessary to ensure antiproliferative property. The ethanol extract showed low mitotic index of 0.34% and whereas the cytolytic index were found to be 80% for ethanol. Teixeira *et al.* [14] have reported that infusions prepared from the medicinal plants *Psidium guajava* L. and *Achillea millefolium* L. showed mitotic index of 1.1% and no activity for *Achillea millefolium L.* which comparatively less than the present investigation. The cell growth recovered in both the plants after 24 hrs treatment whereas there is no recovery in the present analysis.

Recent reports have cited that so many plants and its components could act as tumor suppressor, apoptotic inducers in cancer cells. For example Ginseng from *panax ginseng,* the most commonly used herbal medicine have tumor suppressing activity, interfere with cell cycle progression, enhance immune activity and suppress tumor angiogenesis [15]. Likewise the aqueous extract of *Helixanthera parasitica* is also reported [16]. In the present study the *Amorphophallus* tuber extracts is well correlated with previous reports from different plant extracts on cancer suppressing activity or anticarcinogenic activity.

In conclusion, the results of these investigations should be helpful in the better explaining the complex pharmacological activity of *Amorphophallus* tuber. Following these studies the present study confirms the potential of *A. paeoniifolius* extracts can be used as anticancer drug. Further more mechanistic work is essential to prove these compounds as a one of the specific cancer drug.

REFERENCES

1. Baker, J.T., R.P. Borris, B. Carte, G.A. Cordell and D.D. Soejarto *et al.*, 1995. Natural product drug discovery and development: New perspective on international collaboration. J. Natl. Prod., 58: 1325-1357.
2. Trichopoulos, D. and W.C. Willett., 1996. Nutrition and cancer. Cancer Causes Cont., 7: 3-4.
3. Block, G., 1992. The data support a role for antioxidants in reducing cancer risk. Nutr. Rev., 50: 207-213.
4. Rogers, A.E., S.H. Zeisel and J. Groopman, 1993. Diet and carcinogenesis. Carcinogenesis, 14: 2205-2217.
5. Dorai, T. and B.B. Aggarwal, 2004. Role of chemo preventive agents in cancer therapy. Cancer Lett., 215: 129-140.
6. Barnes, S., 1995. Effect of Genistein on *in vitro* and *in vivo* Models of Cancer. J. Nutr., 125: 777-783.
7. Singh, B., T.K. Bhat and B. Singh, 2003. Potential therapeutic applications of some antinutritional plant secondary metabolites. J. Agric. Food. Chem., 51: 5579-5597.
8. Agrawal, D.P., 2002. Complementary and alternative medicine: an overview. Curr. Sci., 82: 518-524.
9. Knoll, M.F., A.C.F. Da Silva, T.S.D. Canto-Dorow and S.B. Tedesco, 2006. Effects of *Pterocaulon polystachyum* DC. (Asteraceae) on onion (Allium cepa) root-tip cells. Genet. Mol. Biol., 29: 539-542.
10. Tengchaisri, T., R. Chawengkirttikul, N. Rachaphaew, V. Reutrakul, R. Sangsuwan and S. Sirisinha, 1998. Antitumor activity of TPL against cholangiocarcinoma growth *in vitro* and in hamsters. Cancer Lett., 133: 169-175.
11. Heinrich, M. and P. Bremner, 2006. Ethnobotany and ethnopharmacy-their role for anti-cancer drug development. Curr. Drug Targets, 7: 239-245.

12. De Flora, S. and L.R. Ferguson, 2005. Overview of mechanisms of cancer chemopreventive agents. Mutat. Res., 591: 8-15.
13. Howells, L.M. and M.M. Manson, 2005. Prospects for plant-derived chemopreventive agents exhibiting multiple mechanisms of action. Curr. Med. Chem. Anticancer Agents, 5: 201-213.
14. Teixeira, R.D.O., L.C. Marjori, M.S. Mantovani and V.E.P. Vicentini, 2003. Assessment of two medicinal plants, *Psidium guajava* L. and *Achillea millefolium* L., in *in vitro* and *in vivo* assays. Gent. Mol. Biol., 26: 551-555.
15. Sato, K., M. Mochizuki, I. Saiki, Y.C. Yoo, K. Samukawa and I. Azuma, 1994. Inhibition of tumor angiogenesis and metastasis by a saponin of *Panax ginseng*, ginsenoside-Rb2. Biol. Pharm. Bull, 17: 635-639.
16. Lirdprapamongkol, K., C. Mahidol, S. Thongnest, H. Prawat *et al*., 2003. Anti-metastatic effects of aqueous extract of *Helixanthera parasitica*. J. Ethnopharmacol., 86: 253-256.