



Scholars Research Library

Archives of Applied Science Research, 2014, 6 (4):139-142
(<http://scholarsresearchlibrary.com/archive.html>)



The cytotoxicity of different plant extract on chick embryo fibroblast cell line

S. B. Kakad¹ and A. J. Dhembare²

¹Department of Biotechnology, Padmashri Vikhe Patil College of Arts, Sci. & Comm., A/P- Loni, Pravaranagar, MS, India

²Department of Zoology, Padmashri Vikhe Patil College of Arts, Sci. & Comm., A/P- Loni, Pravaranagar, MS, India

ABSTRACT

This present work described the chick embryo cell line fibroblast toxicity on control and induced cells. Observation was made on selected plant extract on fibroblast during 2013-14 under laboratory condition. Twenty plant species were selected for their establishment and three species were found high percent viability such as *Tinospora cardifolia* (82.33%), *Plumbago zeylanica* (76.66%) and *Withania somnifers* (69.81%). Species *Crosandrin fundibulformis* (23.07%) and *Cathranthus rosae* (18%) were reported lowest viability. The viability varies species to species and discussed.

Key words: Cytotoxicity, ethanol extracts, fibroblast, cell line.

INTRODUCTION

A fibroblast synthesizes the extracellular matrix and collagen [1] and plays a critical role in wound healing. Fibroblasts are the most common cells of connective tissue in animals. Animal cell culture became a common laboratory technique in the mid-1900s, but the concept of maintaining live cell lines separated from their original tissue source was discovered in the 19th century. The fibroblasts are often used as feeder cells in human embryonic stem cell research. The toxicants usually are contaminants discharged into the environment through human actions and having the potential to impact on ecosystems at relatively low concentrations [2]. Most often, ecotoxicants arise as a result of industrial activities; with polycyclic aromatic hydrocarbons (PAHs) being one example, but pharmaceuticals released through medical and farming practices should also be considered [3] which shoed effect on cell. Cell lines are used to study and identify new biomarkers and provide experimental insight into their basis. Cell lines have been used extensively to study the cytotoxicity of substances to animal cells [4, 5]. Recently literature showed work on cell line as embryo extract of chick pictorial muscle and myoblast clone [6], myoblast derived from cell line [7], cyto-genotoxicity of organochemicals to cell [8], organophosphrous [9], cancer cell [10,11].

Cell lines are the only regularly available source of biological material for experimentation. Evaluating general cytotoxicity can be done in a variety of ways, which will be referred to as cell viability assays. Aim of the present study was to evaluate different plant extracts cytotoxicity on chick cell line fibroblast. The data may provide additional insight into etiologic and pathophysiologic mechanisms in the autoimmune disorders. Evaluation of cell viability in animal (chick) with autoimmune system may help determine the process of a diseases or change in treatment goals and options. It helps for immunity and indicators of metabolic activity. These extracts are useful for viability and medium for culture in experimental work.

MATERIALS AND METHODS

Plant material:

Healthy plant materials were collected from host college botanical garden. Materials were washed with tap water to remove dust. After washing immersed in tap water with 2-3 drops of Teepol for 10 minutes, then washed with distilled water and sterilized (0.1% HgCl₂ for 3 min) and again washed with sterile distilled water for three times. Sterilization was done in laminar hood. The sterilized materials were cut in to smaller pieces and placed on culture medium supplemented with different concentrations of auxins and cytokinines.

Methanol extract:

Leaves were rinsed with sterilized distilled water and crushed in pure methanol. The extract was filtered through muslin cloth. Filtrate was allowed to settle at room temperature for 2-3 days. Upper methanolic layer was discarded, extract was collected and preserved in cooled condition as crude extract and used for experimental work.

Cytotoxicity activity:

Fibroblast cell line was established from chick embryo using DMEM medium supplemented with serum (Fetal Bovine serum 10% and gentamicine 50µg/ml). A fibroblast cells (5ml) line suspension was added to six well microtitre plates. Different concentrations (50µl, 100µl, 150µl) of leaf methanolic extracts were added to each well in triplicates. The microtitre plate was incubated aseptically in CO₂ incubator for 24 hours at 37°C. After incubation cells were disaggregated using trypsin (0.25%). Percent viability was made using Trypan Blue on Neubauers Chamber. Percent viability was calculated using standard formula [12]. Experiments were carried out in duplicate and each experiment was repeated at least two times. Mean and standard errors were used throughout the study and the values were compared using Duncan's multiple range tests [13].

RESULTS AND DISCUSSION

It was seen from the present study that the viability in the chick embryo cell line was occurred due to treatment of respective extract. The data presented in Table 1, that indicated the percent viability of various plants extracts which were varied plant to plant. It was revealed that high percent viability from *Tinospora cardifolia* (82.33%), *Plumbago zeylanica* (76.66%) and *Withania somnifers* (69.81%). Species *Crosandrin fundibulformis* (23.07%) and *Cathranthus rosae* (18%) were reported lowest viability.

The extract of *Tinospora cardifolia* revealed highest viability which compare to those of standard control. *Tinospora cardifolia* consists of as Tinofend has been studied clinically. One study in 75 patients with allergic rhinitis (hay fever) showed statistically significant reduction of symptoms compared to placebo [14]. An independent review of this study concluded that significant intergroup differences were seen in all symptoms, although studies in larger populations may support this finding [15]. A combination of *T. cordifolia* extract and turmeric extract was effective in reducing the hepatotoxicity which was induced by the combination of isoniazid, rifampicin, pyrazinamide and ethambutol for treating tuberculosis [16]. In the present study, this species revealed high viability result which may be a ingredients present in plant. It is also useful for further media culture.

Plumbago zeylanica showed second rank of viability compare with standard control. Plant extracts have shown potent mosquito larvicidal activity against the larvae of *Aedes aegypti* while showing no toxicity to fish [17]. Hexane extracts of *Plumbago zeylanica* have shown activity against canine distemper virus [18]. Hexane extract of *Plumbago zeylanica* Plumbagin shows antimicrobial activity [19]. Methanolic extract of *Plumbago zeylanica* positive inotropic activity [20]. Enzymatic spectrum of herbal plants Plumbago was carried out [21]. Bioactive spectra of Plumbagin methanol extract of *Plumbago zeylanica* shows effect on root-knot nematode *Meloidogyne* spp. [22,23]. This indicated that *P. zeylanica* is useful in insecticide, antimicrobial, nematocidal activities and it is also useful in cell line culture.

Withania somnifera was showed the third rank in the viability test. The main chemical constituents are alkaloids and steroidal lactones. These include tropine and cuscohygrine. The leaves contain the steroidal lactones, withanolides, notably withaferin, which was the first to be isolated from the plant. *Withania somnifera* is prone to several pests and diseases. It is also reported high percent viability in cell line and useful in cell line medium.

At prime phase of viability in conducted experiment all extract have not equal viability; some had more while some least. These extract have some other properties such as- insecticidal, antibacterial, antiviral, antifungal, etc properties. But in the present investigation the scope was made only for viability on fibroblast. Author's are further planned to study the antibacterial, antifungal, etc. activity of the highly viability showed plant species.

Chick embryo cell line likely will become increasingly utilized in toxicology, but further developments will be needed to maximize their potential. The technological needs are in several interconnected areas in the science of culturing chick cells. These include understanding their nutritional requirements, differentiation capacity, direct immortalization, cell lineage position and transfection. The full value of chick cell lines will be realized when more ecotoxicologists are willing to view cell lines as one of the many complementary approaches to explore the complexity of animal and to place concerns about the normalcy of cell lines in a realistic perspective. Cell lines can never rival the beauty and diversity of animal species but in conjunction with other approaches they should greatly contribute to the acquisition of knowledge about this marvelous group of higher animals and help to understand the impact of toxicants on them.

Table 1: Showing effect of different plant extract on chick embryo fibroblast cell line.

Sr No	Name of the plant species	% viability
1	<i>Tinospora cordifolia</i> (Thumb)	82.33
2	<i>Withania somnifera</i> (Lin)	69.81
3	<i>Achyranthes aspera</i> (Lin)	52.56
4	<i>Rauvolfia tetraphylla</i> (Lin)	50.54
5	<i>Clematis gouriana</i> (ROXB)	55.35
6	<i>Datura stramonium</i> (Lin)	60.50
7	<i>Tribulus terrestris</i> (Lin)	25.00
8	<i>Cleome viscosa</i> (Lin)	33.33
9	<i>Catharanthus roseus</i> (Lin)	38.23
10	<i>Ageratum conyzoides</i> (Lin)	40.67
11	<i>Argemone mexicana</i> (Lin)	37.97
12	<i>Lantana camara</i> (Lin)	35.84
13	<i>Tephrosia purpurea</i> (Lin)	42.10
14	<i>Tridax procumbens</i> (Lin)	26.92
15	<i>Sida acuta</i> (Lin)	29.41
16	<i>Vitex negundo</i> (Lin)	25.80
17	<i>Crossandra in fundibuliformis</i> (Lin)	23.07
18	<i>Catharanthus roseus</i> (Lin)	18.01
19	<i>Plumbago zeylanica</i> (Lin)	76.66
20	<i>Boerhavia diffusa</i> (Lin)	84.37
21	Methanol control	64.00

REFERENCERS

- [1] P. Weissmanshomer and M. Fry, *Mechanisms of Ageing & Development* **1975**, **4** (2):159-166.
- [2] D. Connell, P. Lam, B. Richardson and R. Wu, *Introduction to Ecotoxicology*, Oxford, England, Blackwell Science, **1999**.
- [3] O. A. H. Jones, *Environ. Technol.* **2002**, **22**: 1383-1394.
- [4] H. Babich and E. Borenfreund, *Toxicol.* **1991**, **5**: 91-100.
- [5] H. Segner, Fish cell lines as a tool in aquatic toxicology. In: *Ecotoxicology*, Edited by T. Braunbeck, D.E. Hinton and B. Streit, Basel, Birkha user, **1998**, pp. 1-38.
- [6] T. P. Nowak, P.L. Haywood, S.H. Barondes, *Biochem. & Biophysical Res. Communi.*, 1978, **68** (3): 650-657.
- [7] Bernardo Nadal-Ginard, *Cell*, **1978**, **15** (3): 355-364.
- [8] H. Babich, S.H. Goldstein and E. Borenfreund. *Toxicol. Lett.* **1990**, **50**: 143-149.
- [9] Li, H. and S. Zhang. *Toxicol. In Vitro*, **2001**, **15**: 643-647.
- [10] C. Glenn Begley and Lee M. Ellis, *Nature*, **483**, 531-533.
- [11] Robert H. Shoemake, *Nature Reviews Cancer*, **2006**, **6**, 813-823.
- [12] R. I. Freshney, *Culture of Animal Cell: A Manual of Basic Techniques*, 5th Ed, John Wiley & Sons Inc. **2005**.
- [13] K. Gomez, and K. A. Gomez, *Statistical procedures for agricultural research with emphasis on rice*. Los. Banos. International Rice research **1976**.

- [14] V. A. Badar, V. R. Thawani, P. T. Wakode, M. P. Shrivastava, K. J. Gharpure, L. L. Hingorani and R. M. Khiyani, *J. Ethnopharmacology*, **2005, 96** (3): 445-449.
- [15] R. Guo, M. H. Pittler, and E. Ernst, *Annals of Allergy Asthma & Immuno.* **2007, 99** (6): 483-495.
- [16] M. R. Adhvaryu, M. N. Reddy, and B. C. Vakharia, *World J. Gastroenterology*, **2008, 14** (30): 4753-4762.
- [17] C. D. Patil, S. V. Patil, B. K. Salunke, R. B. and Salunkhe, *Parasitol. Res.* **2011, 108** (5):1253-63
- [18] V. P. Bagla, L. J. McGaw and J. N. Eloff, *Vet. Microbiol.* **2011**, Sep 17;
- [19] L. B. Dama, B. N. Poul and B. V. Jadhav, *J. Ecotoxicol. Environ. Monit.* **1998, 8**:213-215.
- [20] B. N. Poul, L. B. Dama and B. V. Jadhav, *J. Sci. Engineering Res.* **1999, 11**: 26-29.
- [21] B. N. Poul, L. B. Dama and B. V. Jadhav, *Asian J. Chem.* **1999,11** (1):144-148.
- [22] B. N. Poul, L. B. Dama and B. V. Jadhav, *Asian J. Chem.* **1999, 11**(1):273-275.
- [23] L. B. Dama, *Indian Phytopatho.***2002, 55** (1): 67-69.