

Cytotoxicity Study in Non Malignant Fibroblast L929 Cell Line with *Mucuna pruriens* Seed Extract

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Abstract

Mucuna pruriens (L.) DC. (Leguminosae), commonly called velvet bean has been long used by tribes as an antidote for various snakebite, for treating depression and various nervous disorders, Parkinson's disease, arthritis and also has potent anti-urolithiatic, anti-diabetic, anti-cancer, anti-oxidant activities. To evaluate the potential cytotoxicity of the hydroalcoholic extract of *Mucuna pruriens* seeds in non-malignant fibroblast L929 cell line. Main Findings: To access the toxic nature of the seeds, a hydroalcoholic extract was prepared and tested for its cytotoxic effects on non-malignant fibroblast L929 cell line at various concentrations (1.25 mg/ml, 2.5 mg/ml and 5 mg/ml). Viability (%), cell death (%), cytotoxicity grading, proliferation rate and growth inhibition values were calculated. No substantial difference in viability (%), cell death (%), growth inhibition (%) and proliferation rate were observed either at 24 h or 48 h following treatment with various concentrations of the extract as compared to the negative control. No toxicity was observed at any concentration of the extract, implying that the L-DOPA enriched extract of *Mucuna pruriens* is not toxic to the non malignant cells. These results confirmed that *Mucuna pruriens* seeds are safe and can be used as medicine for the treatment of various diseases.

Keywords: *Mucuna pruriens* seeds, L-DOPA enriched extract, L929 cell line, Toxicity

Introduction

Mucuna pruriens (L.) DC. (Commonly called velvet bean) is a tropical legume well acknowledged for its versatile beneficial properties. It is one of the effective mood enhancing and libido stimulating [1] super foods available and used as food supplement for depression, various nervous disorders and also in arthritis [2-4]. In 2002, a U.S. patent was filed on the use of velvet bean to stimulate the release of growth hormone in humans. The main medicinal property attributed to *Mucuna pruriens* come from the seeds, but the pod and roots can also be used in herbal preparations. The seeds of *Mucuna pruriens* have been used in traditional Indian medicine for treating diseases including Parkinson's disease [5]. The mature seeds of the plant contain about 3.1–6.1% L-DOPA [6-7] with various other mind-altering chemicals, including serotonin (5HT), oxitriptan (5-HTP), nicotine, N,N-DMT, bufotenin (5-HO-DMT) and 5-MeO-DMT. The ethanolic extract of leaves of *Mucuna pruriens* has anticataleptic and antiepileptic effect in albino rats. Dopamine and serotonin may have a role in such activity [8]. Apart from being anti urolithiatic [9], the anti-diabetic, anti-cancer and anti-oxidant properties have also been reported from this plant [10-12]. The plant and its extracts have been long used by tribes as an antidote for various snakebites [13-14]. If consumed in large quantities as food, crude *Mucuna pruriens* is poisonous to mammals [15].

This suggests that toxicity is the main issue linked to *Mucuna pruriens* therefore, in an attempt to obtain a non toxic preparation, in the present article; we prepared L-DOPA enriched extract of *Mucuna pruriens* using Amberlite cation exchanger and assessed its toxicity in non malignant fibroblast L929 cell line at various concentrations (1.25 mg/ml, 2.5 mg/ml and 5 mg/ml) under in vitro conditions.

Material and Method

Plant Material

The seeds of *Mucuna pruriens* were gifted by Patanjali Ayurveda Ltd., Haridwar, India and stored in ambient conditions for further study. The other solvents and chemicals were purchase from Sigma-Aldrich and S.D. Fine Chemicals, India. The non-malignant fibroblast L929 cell line was obtained from National Centre for Cell Science (NCCS), Pune, India.

Preparation of Hydroalcoholic Extract

The powdered seeds of *Mucuna pruriens* (100 gm) were subjected to extraction using Methanol: Water (60:40). The extract was evaporated to dryness in a rotary flash evaporator at a temperature not exceeding 60 C, then stored in air tight container. The concentrate was passed through HCl treated Amberlite cation resin. The resin was washed thoroughly with Ammonium hydroxide and concentrated to obtain L-DOPA enriched extract.

Preparation of Test System

The non-malignant fibroblast L929 cell line was set up in bulk culture flask containing culture medium. Cell line of passage number 5 was used to prevent use of cells undergoing genetic drift and other variations. The cell line was incubated at 37+2oC in humidified 5% CO₂ and 95% air atmosphere. Bulk cultures were sub-cultured in required number of T 25 flask after achieving approximately 80% confluence. Bulk culture was trypsinized and cell count of the cell suspension was performed in triplicate. Mean value of the same was considered for cell culture initiation for the study. The cell line was incubated for two days before the treatment to achieve approximately 80% confluences at 37+2oC in humidified 5% CO₂ and 95% air atmosphere as appropriate for the buffer system chosen for the culture medium. Cell line from passages was cryopreserved as stock culture in cryovials with freezing media (20% serum containing culture medium+ 10% v/v DMSO) in liquid Nitrogen. This served as the cell bank for the experiment. The cell line was discarded and replaced with new lines from the bank after every 10 passages.

Test System acceptability

Before treatment, cell cultures were examined for the acceptability of the culture in the study. Passage number for the cell line used in the study was between P-3 to P-10 to ensure the authenticity of the test system as well as reliable and reproducible results. The viability of the seed culture was determined by trypan blue dye exclusion assay and it was more than 95%.

Experimental protocol

The in vitro cytotoxicity experiment was performed by direct deposition method. In the experiment, the cytotoxicity was evaluated in comparison to negative control using in vitro cytotoxicity test. As per the direct-deposition method the cells were exposed to various concentrations of the extract (1.25 mg/ml, 2.5 mg/ml and 5 mg/ml) to establish a dose response effect, and after 24 and 48 h of incubation the viable and non viable cell count of the original single cell suspension, % viability, % cell death or % damaged cells, proliferation rate and % growth inhibition were scored. Based on the solubility and precipitation test, Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum was selected for the study. Concurrent negative control (culture without any treatment) was performed. Phenol solution (0.2%) in PBS was used as positive control. The formulas used for calculation are:

Viable cells/ml = Total viable cells counted in 4 squares x Dilution factor x 10⁴

Dead Cells/ml = $\frac{\text{Total dead cells counted in 4 squares} \times \text{Dilution factor}}{4} \times 10^4$

% Viability = $\frac{\text{Total number of viable cells} \times 100}{\text{Total number of cells}}$

% Cell Death (% Damaged cells) = 100 - % Viability
Proliferation rate (pR) = $\frac{\text{Number of cells at time t (Nt)}}{\text{Number of cells at time to (No)}}$

No = Cell count at time of seeding, Nt = Cell count at the time of harvesting

% Growth inhibition = $100 - \left[\frac{\text{Number of cells of treated flask}}{\text{Number of cells of control flask}} \right]$

Total number of cells in the original single cell suspension = [Cell/ml x Volume x Dilution]

Results and Discussion

All flasks (negative control, positive control and test item treatment) were analysed after incubation for evidence of cytotoxicity which was judged by direct microscopic examination. The results were evaluated on the basis of viability (%), cell death (%), and proliferation rate and growth inhibition (%). Visual scores were assigned to the test item and controls using a rating scale (0-3). The investigation revealed a high degree of correlation between visual cytotoxicity rating and quantitative cell viability measurements. No substantial difference in viability (%), cell death (%), growth inhibition (%) and proliferation rate were observed either at 24 h or 48 h following treatment with various concentrations of the extract (1.25 mg/ml, 2.5 mg/ml and 5 mg/ml) when compared to the negative control (Table 1). No noticeable increase in the cell death was observed as the cells displayed comparable results to the negative control (Figure 1a & 1b). Thus, both negative control and test item treatment groups, elicited non-toxic response and received score of 0 at 24 a and 48 h grading intervals in L929 cell line that displayed discrete intercellular granules with occasional cell lysis. However, positive control (Phenol, 0.2%), received a score of 3 at 24 and 48 h grading intervals with the cell type that displayed a sever response with complete destruction of cell monolayer. Cells showed signs of cytotoxicity, rounding of the cells and increased spacing between the cells. As the time progressed, the cytotoxic effects became more prominent until complete destruction of cell monolayer at 48 h. The above results conclude that *Mucuna pruriens* seed extract (L-DOPA enriched) is non-cytotoxic to non-malignant fibroblast L929 cell line.



Figure 1 -(a) Proliferation Rate (pR) and (b) Growth inhibition (%) at 24 h and 48 h Harvesting at Three Different Concentrations of the Extract (T1=1.25 mg/ml, T2=2.50 mg/ml, T3=5.00 mg/ml) Compared to Negative and Positive Controls

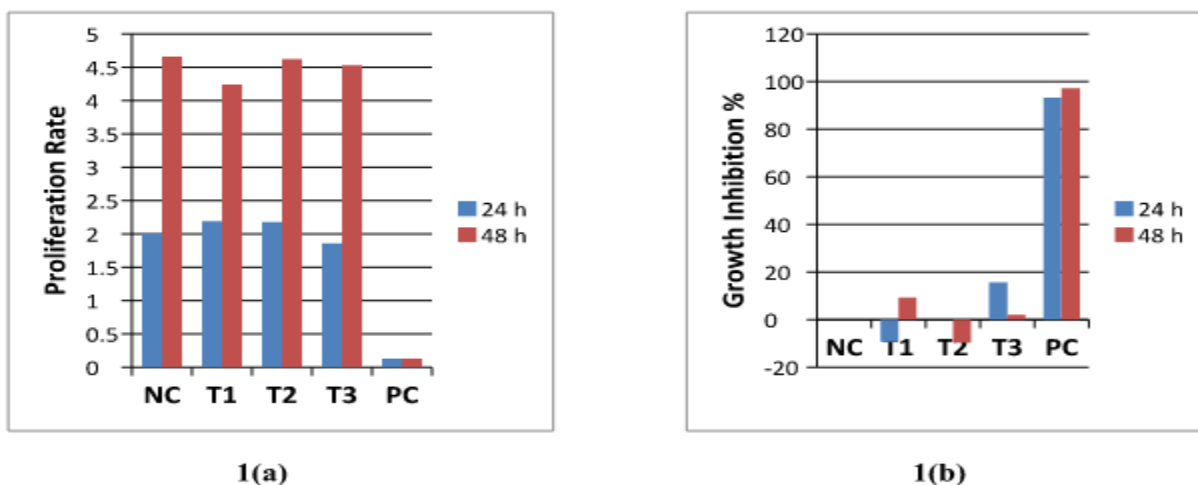


Table 1-Mean Viability(%), Cell Death (%), Growth inhibition (%) and Proliferation Rate (pR) at 24 h and 48 h Harvesting at Three Different Concentrations of the Extract (T1=1.25 mg/ml, T2=2.50 mg/ml, T3=5.00mg/ml)

Dose	24 hour				48 hour			
	Viability (%)	Cell death (%)	pR	Growth inhibition (%)	Viability (%)	Cell death (%)	pR	Growth inhibition (%)
NC	95.90	4.10	2.00	0	94.80	5.20	4.66	0
T1	88.51	11.50	2.19	-9.37	95.27	4.73	4.24	9.39
T2	93.41	6.60	2.18	0	95.46	4.54	4.62	-9.62
T3	93.67	6.33	1.86	15.71	96.45	3.54	4.53	2.02
PC	14.55	85.44	0.13	93.22	8.09	91.91	0.13	97.24

Data expressed as mean±SD, N=3

Conclusion

L-DOPA enriched extract of *Mucuna pruriens* was prepared and assessed for biological reactivity using non-malignant fibroblast L929 cell line at various concentrations (1.25 mg/ml, 2.5 mg/ml and

5 mg/ml). Viability (%), cell death (%), cytotoxicity grading, proliferation rate and growth inhibition values were calculated. The pR and GI values indicated that there is no toxicity at any concentration of the extract, which implies that the L-DOPA enriched extract of *Mucuna pruriens* is non-toxic to non malignant



cells. These results confirmed that *Mucuna pruriens* seeds are safe and can be used as medicine for the treatment of various diseases.

Authors' contributions

ABK and HKM have made substantial contributions to conception and design and acquisition of data. VKS and NS involved in analysis and interpretation of data, drafting the manuscript and revising it critically for important intellectual content.

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Conflict of Interest

The authors declare no conflict of interest.

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