Evaluation of Cytotoxicity of *Gymnema sylvestre* **on** L929 Mice Fibroblast Cell: An *In Vitro* Study

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Abstract

Background: Cytotoxicity is defined as the toxicity caused due to the action of chemotherapeutic agents on living cells. Cytotoxicity tests are very important as they help in the determination of the proposed biomedical use. It has been proven that these cytotoxic properties of the medicinal plants are mainly due to its phytochemical constituents. Gymnema sylvestre is one such traditional ayurvedic herb used primarily for the treatment of diabetes and other ailments such as arthritis, anemia, diuretics, etc. Aim: The aim of this study is to analyse the cytotoxic property of ethanolic extract of Gymnema sylvestre in comparison with the sodium hypochlorite. Materials & methods: The ethanolic extract of Gymnema sylvestre was prepared and the cytotoxic potential of the extract at different concentrations (25,50,100,200 µg/ml) was tested on L929 mice fibroblast cell using MTT assay and compared with that of the standards sodium hypochlorite (NaOCl). Results: It is shown that the ethanolic extract did not adversely affect the fibroblasts even up to 50% concentration showing a nontoxic effect even till 200 µg/ml dose in comparison with NaOCl on these cells. Conclusion: Within the limitations of this study it can be concluded that ethanolic extract of *Gymnema sylvestre* did not adversely affect the fibroblasts even up to 50% concentration showing a nontoxic effect even till 200 µg/ml dose in comparison with NaOCl on L929 mice fibroblast cells. However, further preclinical and ex vivo studies have to be carried to prove its use in clinical trials.

Keywords: Cytotoxicity; Gymnema sylvestre; MTT assay; L929 Mice fibroblast cells

Introduction

Natural products have been used since ancient times for the treatment of many diseases especially at the primary health care level in developing countries like India.

Due to the increase in resistance to the commercially available drugs there is a great interest in the search for new antimicrobial drugs also from nature. Natural crude drug extracts and biologically active compounds isolated from plant species used in traditional medicine can be prolific resources for such new drugs. ^[1–3]

The 70% of the drugs used today are models of natural products which were obtained from the leaves, barks and roots of medicinal plants. ^[4] Most of the commercially available drug has an active ingredient derived from a plant

Thus in this technological age; plants continue to play a significant role both medically and economically. ^[5,6] The significance of these medicinal plants in treating various ailments can be attributed to bioactive constituents who are used directly as drugs or pharmacological agents.

Thus these components have to be assessed for their cytotoxic effect on the living cells before their use.

Cytotoxicity is defined as the toxicity caused due to the action of chemotherapeutic agents on living cells. Cytotoxicity tests are very important as they help in the determination of the proposed biomedical use.^[7]

Gymnema sylvestre is a perennial woody vine native to tropical Asia, China, Africa and Australia. It has been widely used in ayurvedic medicine for treating various illnesses.

This exhibit a broad range of therapeutic effects for various conditions such as diabetes, arthritis, diuretics, anemia, hypercholesterolemia, etc. It also shows antibacterial, anti-inflammatory and anti-carcinogenic properties.^[8–10]

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Methodology

Chemicals

The chemicals used for the MTT test were 3-(4,5dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide, 10% Fetal Bovine Serum (FBS, 100 units/ml of penicillin, Dimethyl Sulfoxide (DMSO, human fibroblast cell lines (primary culture, Eagle's Minimum Essential Medium (EMEM, kanamycin, and phosphate-buffered saline.

Maintenance of cell lines

L929 fibroblast cell lines were purchased from NCCS Pune. The L929 Cells were cultured in a humidified atmosphere at 37°C in the cell growth DMEM medium with 10% fetal bovine serum, L–glutamine, 1% penicillin (100 U/ml, and streptomycin (100 μ g/ml) at 37°C in a humidified CO₂ (5%) chamber and 95% air.

The cells were detached using 0.25%EDTA Trypsin. Neutralization of the Trypsin was achieved using DMEM containing 10% FBS and PSGF, and cells were mechanically separated using a pipette.

There were 96-well plastic culture plates filled with 200 μ l of medium containing each well. The plates were then incubated at 37°C in a humidified atmosphere containing 5% CO₂ and 95% air for 24 hrs to permit attachment of the cells to the plates.

Cell viability by MTT assay

For cell viability assay, the microplates filled with 100 μ l of L929 cells with a density of 1×10^5 as negative control. The cells were permitted to adhere for 24 hrs, and the growth medium using micropipette and the monolayer of cells washed twice with MEM without FBS to remove dead cells and excess

FBS. About 1 ml of medium (without FBS containing different dilution of *Gymnema sylvestra* extract (25,50,100,200 μ g/ml and for comparison 2.5% NaOCl were added in respective wells; 20 μ l of MTT (5 mg/ml in PBS)were added to each well, and the cells incubated for a further 6-7 hrs in 5% CO₂ incubator.

After removal of the medium, 1 ml of DMSO was added to each well and tested. The supernatant was removed and 50 μ l of propanol was added and the plates were gently shaken to solubilize the formed formazan.

The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The plates were placed on a shaker for 15 min and the absorbance was read on an Enzyme-Linked Immune Sorbent Assay (ELISA)(MINDRAY90) reader at 570 nm.

Each experiment was carried out in triplicate and the IC50 of the test samples as the percentage survival of the cells was calculated.^[11,12]

Statistical analysis

Results were expressed as mean \pm S.E.M. Statistical significance was determined by one-way Analysis Of Variance (ANOVA) and post hoc least-significant difference test by SPSS software (version 22.0). P values less than 0.05 were considered significant.

Results and Observations

MTT assay absorbance value of L929 cells after the treatment with *Gymnema sylvestra* ethanolic extract showed that the extract did not adversely affect the fibroblasts even up to 50% concentration showing a nontoxic effect even till 200 μ g/ml dose in comparison with NaOCl on these cells [Table 1, Figure 1].

Table 1: Showed the value of L929 cells after the treatment with Gymnema sylvestra ethanolic extract.			
S. No.	Treatment	Conc. (µg/ml)	Mean ± SEM
1	L929 untreated cells	-	0.513 ± 0.04
2	GSE	25	0.489 ± 0.40a
3		50	0.436 ± 0.35a
4		100	0.424 ± 0.22a
5		200	0.409 ± 0.33*a
6	NaOCI	2.50%	0.226 ± 0.24*

Values are expressed as Mean ± SEM (n=3); *: p<0.05; p<0.001, as compared with negative control. ap<0.001, as compared with NaOCI. The IC50 of the extract is more than 200 µg/ml.



Figure 1: Showed the value of L929 cells after the treatment with *Gymnema sylvestra* ethanolic extract. Values are expressed as Mean \pm SEM (n=3); p<0.001 statistically significant as compared with negative control. However, GSE did not adversely affect the fibroblasts even up to 50% concentration showing a nontoxic effect even till 200 µg/ml dose in comparison with NaOCl on these cells.

Discussion

According to preventive medicine, Plants are considered as a potential source of new antioxidants which have replaced the synthetic antioxidants due to safety concerns. ^[13] Gymnema sylvestre which is primarily used for its antidiabetic property, possess various phytochemicals such as flavonoids, tannins, alkaloids, phenols, ascorbic acid, folic acid etc. [14-16] Research has proved that specific photochemicals are responsible for the specific activity such as flavonoids has antioxidant potential while alkaloids have antimicrobial, analgesic, and other antispasmodic actions and steroids have inflammatory potency. ^[17] A study by Rahman et al. has shown that the ethanolic extract of Gymnema sylvestre has antioxidant potential and also its potential is better than that of A. bilimbi and C. frutescens.^[18] The antioxidant activity of Gymnema sylvestre was also investigated by Rupanar and Ohmori against butylatedhydroxytoluene, proving it possesses better DPPH scavenging activity and also to reduce LDL oxidation.^[19]

In another study, showed the scavenging activity of hydroxyl free radical and antioxidative potential of this plant. ^[20] Studies have proven that the extract have antioxidant potential in conditions high fat diet, hydrogen peroxide, nitric oxide and superoxide radical induced oxidative stress in rats. ^[21–24] Rachh et al. has shown that the methanolic extract of *Gymnema sylvestre* having significant radical scavenging activity against ferric, super oxide, hydrogen peroxide. Previous studies have shown the efficacy of methanolic extract of *Gymnema sylvestre* as potent antioxidant but there are limited studies using its ethanolic extract. ^[25]

According to the results of this study, the ethanolic extract of *Gymnema sylvestre* showed that fibroblast cells were not adversely affected even till the concentration of 200 μ g/ml. This result suggests that ethanolic extract of *Gymnema sylvestre* has non toxic effect on the fibroblasts cells recommending its use as alternative to synthetic drugs.

Conclusion

Within the limitations of this study it can be concluded that ethanolic extract of *Gymnema sylvestre* did not adversely affect the fibroblasts even up to 50% concentration showing a nontoxic effect even till 200 μ g/ml dose in comparison with NaOCl on L929 mice fibroblast cells. However, further preclinical and *ex vivo* studies have to be carried to prove its use in clinical trials.

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