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Cytotoxicity Evaluation of *Swasanandam Gutika* by MTT Assay in Mouse Fibroblast Cells

Sugeena P[1], Tharalakshmi S[2]

[1]Assistant Professor, Department of Rasasastra and Bhaishajyakalpana, KMCT Ayurveda Medical College, Manasserry, Kozhikode, Kerala
[2]Associate Professor, Department of Rasasastra and Bhaishajyakalpana, Government Ayurveda College, Thiruvananthapuram, Kerala

Corresponding Author: Sugeena P

ABSTRACT

Introduction: Swasanandam gutika (SG) is one among the popular rasa formulation mentioned in Arogyakalpadruma, a textbook on balachikitsa. SG is a pill formulation containing Hingula, Vatsanabha and karpoora levigated in Triphalakwatha. Both hingula and vatsanabha are toxic drugs included in the schedule E1 of Drug and cosmetic act 1940. Also camphor is reported to be having toxicity effects. SG is generally administered to children in respiratory illness and hence there is a need of toxicity profiling to ensure safety. Heavy metals like mercury are reported to be having cytotoxic effect. So to evaluate the cytotoxicity of this formulation is an important matter of concern. Aims and objectives: To evaluate the in-vitro cytotoxicity of Swasanandam gutika on mouse fibroblast cells.
Materials and methods: SG was prepared and cytotoxicity was assessed by MTT assay in L929 cell line.
Results: The cell viability at different concentrations are measured. The viability of cells was found to be reduced on increasing the concentration of the drug.
Conclusion: It can be concluded that the drug is safe and non-cytotoxic at concentration less than 125µg/ml.

Key Words: Swasanandam gutika, In-vitro cytotoxicity, MTT assay, L929 cells

INTRODUCTION

It is well said that everything in this world is medicine and it is the correct dose that differentiates a medicine from a poison. Even though the metals and minerals are used as medicine in Rasasastra, they were used in doses of milligram level to achieve therapeutic benefits. Although scientists have expressed concerns on the use of heavy metals in the formulations and possible resultant toxicity, Ayurvedic practitioners insist that calcinations or use of metals such as mercury in particularly their sulfide form abolishes metal toxicity. [1]

Though mercury is known to be toxic in living tissues, salts form of mercury have been traditionally considered nontoxic. Researchers reported that mercury sulfide in traditional medicine has 5000 times less toxic than organic methyl mercury. [2] The efficacy and safety aspects of mercurial preparations have been assessed. [3]

When a new drug, either natural source or synthesized, is under investigation needs to examine its safety to the host cell or the cytotoxic effect in cancer cell. This is well-known as the cell viability test. The assessment of cell viability is based on various cellular functions such as enzyme activity, cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. Among them tetrazolium (MTT) is one of the most frequently methods. This method

uses colorimeter to determine the cell viability. [4] The MTT reagent yields low background absorbance values in the absence of cells. In MTT assay, the linear relationship between metabolically active cells and the color produced is established, thus allowing an accurate quantification of changes in the rate of cell death or proliferation. [5] MTT is the commonly applied method for evaluation of cell viability and cytotoxicity for screening the drugs. The MTT assay based on the reduction of MTT (yellow colored) and other tetrazolium dyes depends upon cellular metabolic activities due to NAD(P) H-dependent cellular oxidoreductase enzymes. [6]

The formulation under study is Swasanandam gutika which is a popular yoga explained in Arogyakalpadruma [7] This book is a valuable literary resource dealing with the paediatric care. This book has been compiled by a great scholar named Kaikulangara Rama Varier who lived in the Kerala terrain in the last century. This literary resource is the one and only leading light in the ancient kerala tradition of Ayurvedic paediatric care. This Yoga was explained in the sixth chapter of this book. SG is a pill formulation containing Hingula, Vatsanabha and karpoora levigated in Triphalakwatha. Both hingula and vatsanabha are toxic drugs included in the schedule E1 of Drug and cosmetic act 1940. Also camphor is reported to be having toxicity effects. This yoga is widely practiced in Kerala by physicians especially in the treatment of respiratory ailments. It is a very familiar yoga among the practitioners and considered to be a better remedy for respiratory disorders. The toxicity profile of this drug is not yet investigated and hence it will be the need of the era to conduct such studies to ensure safety of Rasa preparations. The study aims to evaluate the cytotoxicity of *Swasanandam gutika* by in-vitro MTT assay.

MATERIALS AND METHODS

It is a *Khalweeya rasayoga* prepared by trituration. The formulation contains *hingula, vatsanabha* and *karpoora*. The medium for trituration is *vara rasa, ie triphalakwatha*. The size and dose of the gutika is not explained in the yoga. After preparing pills, it is said to be dried in shade. It is advised to be administered with *sita* (sugar) and *stanya* (breast milk) This gutika is indicated in *kasa, rajayakshma, hikka and swasa.*

Test drug preparations

It comprises the following steps;

1.Sodhana of *Hingula (Mercuric sulphide)*

2.Sodhana of *Vatsanabha (Aconitum ferox)*

3.Preparation of *gutika*

1. Sodhana of hingula

Hingula was purchased from a genuine raw material store from Kollam district. The percentage elemental composition was evaluated by analytical methods to assure the quality. Hingula was weighed and it was placed in a mortar and pestle and powdered. It was triturated with ardraka swarasa for 1 yama (3 hours).The process was repeated 7 times.

Hingula was having a dark red colour with a silvery line. It was changed to bright saffron red and fine powdery texture with a characteristic smell of ardraka.

2. Sodhana of Vatsanabha

Aconitum ferox was procured from SKM sidha and ayurveda company Erode.The method adopted for sodhana procedure was swedana with cow's milk in Dolayantra for 1 yama. Vatsanabha was weighed (62g) and then cut in to small pieces and placed in a pottali. An iron rod was placed over the neck of a mud pot so as to suspend the pottali. 750ml of milk was taken in a mud pot and the pottali was immersed in it without touching the sides and bottom of the vessel. It was kept on the stove and subjected to swedana. After one and a half hours the level of milk in the pot was reduced and it was replaced by an additional 250ml of boiled milk. After 3 hours of swedana the pottali was taken out and

washed in warm water so as to remove the adhered milk scum and other impurities. Then it was kept in sunlight to dry for 10 days.

Changes occurred on performing sodhana

1. The Vatsanabhi pieces were soft and slippery to touch.
2. The quantity of milk was reduced to 560 ml.
3. The colour of milk was changed to light chocolate brown with a characteristic smell.
4. On drying, the weight was reduced by 10g.

3. Preparation of gutika

The preparation of gutika involved three steps – preparation of triphala kwatha for bhavana and trituration of the drugs and rolling of pills.

Preparation of triphala kwatha

The method of preparation of bhavana kwatha explained in Rasatarangini was followed. The quantity of triphala was taken equal to the quantity of drugs. It was then washed and crushed and placed in a mud pot. Eight times of water was added to it and reduced to 1/8. It was filtered and kept aside.

Table 1.Details of Triphalakwatha for Bhavana

Quantity of triphala	Quantity of water	Quantity of kwatha obtained
30g	240 ml	30 ml

Trituration of drugs

10g each of sodhita hingula, sodhita vatsanabha and karpoora was taken. 30 ml of triphalakwatha was obtained. Sodhita vatsanabha and karpoora were powdered and triturated well by adding sufficient quantity of triphala kwatha. After proper grinding karpoora was added and triturated well. It was subjected to three bhavana in triphala kwatha. Each time freshly prepared kwatha was used for bhavana.

Rolling of pills

After three bhavana with triphala kwatha, the grounded mass was rolled in to pills of 120mg weight. Pills were dried in shade and after proper drying, were kept in air tight glass bottles.

Evaluation of cytotoxicity

In vitro cytotoxicity of Swasanandam gutika was evaluated by MTT assay. It is a tetrazolium dye assay which is widely employed to explore the action of drugs on cell viability. Thus the assay measure the metabolic activity of the cells to reduce yellow coloured tetrazolium salt 3-(4,5-Dimethyl thiazol- 2-yl)-2,5-diphenyltetrazolium bromide to purple colored formazen. The study was conducted at Sri Chitra Tirunal Institute for Medical Sciences and Technology, Poojappura, Trivandrum.

The assay was done using L-929 cell lines. This is the fibroblast cell line obtained from adipose tissue of mouse. The cell line was purchased from ATCC. L-929 is an established and well characterised mammalian cell line that has demonstrated reproducible results.

Since the formulation was not soluble in cell culture medium, dimethyl sulphoxide (DMSO) was used as the solubilizing agent. Cell cultured in normal medium was considered as cell control and DMSO processed and diluted similar to test sample was considered as reagent control. A 96 well plate was used to culture the cells by adding culture medium.

The MTT assay was performed to measure the metabolic activity of cells to reduce tetrazolium salt 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide to purple coloured formazen. Test sample was prepared by dissolving 50mg of Swasanandam gutika in DMSO and filtered using 0.22µm membrane filter.10µl of this was diluted to 1ml with culture medium containing serum (500µg/ml) and serial dilutions were prepared using culture medium to get, 250µg/ml, 125µg/ml, 72.5µg/ml, and 36.25µg/ml. Cells cultured in normal medium was considered as cell control and DMSO processed and diluted similar to test sample was considered as reagent control. Equal volume (100µl) of various dilution of test samples, extract of

negative control, cell control, and re-agent control (DMSO in culture medium) were placed on sub-confluent monolayer of L-929 cells. After incubation of cells with various concentration of test sample and controls at 37±1^{0}C for 24±2 hours, extract and control medium was replaced with 50µl MTT solution (1mg/ml in medium without supplements), wrapped with aluminium foil and were incubated at 37±2^{0}C for 2 hours. After discarding the MTT solution 100µl of Isopropanol was added to all wells and swayed the plates. The colour developed was quantified by measuring absorbance at 570 nm using a spectrophotometer. The data obtained for test sample were compared with the cell control.

RESULT

The MTT assay of L929 cells after 24 hour contact with 500µg/ml, 250µg/ml, 125µg/ml, 72.5µg/ml, and 36.25µg/ml of Swasanandam gutika showed 62.82%, 73.03%, 102.29%, 108.21% and 109.84% metabolic activity respectively. Re agent control (DMSO) showed 90.82%, 93.60%, 98.57%, 98.97% and 100.36% activity for 100%, 50%, 25%, 12.5% and 6.25% respectively.

It was evident in the study that the lower concentrations of the gutika (36.25µg/ml) increased the cell viability up to 109.84%. The cell viability decreased below 100% at a concentration above 250µg/ml. Thus it was proved that the concentration of the drug was directly proportionate to the cytotoxicity.

Table 2. Concentration of swasanandam gutika and cell viability

Concentration of gutika in µg /ml of DMSO	Cell viability in percentage
500	62.82
250	73.03
125	102.29
72.5	108.21
36.25	109.84

Table 3. Concentration of solublizing agent DMSO and cell viability

Percentage of dilution of DMSO	Percentage of cell viability
100	90.82
50	93.60
25	98.57
12.5	98.97
6.25	100.36

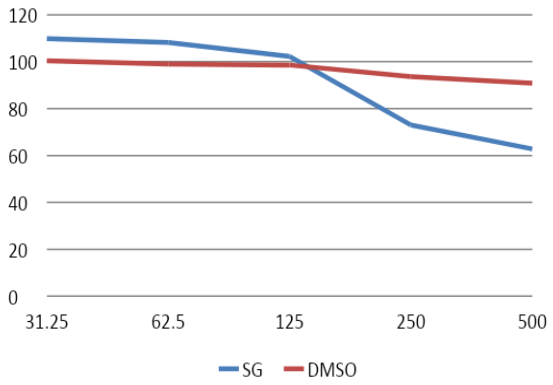

Figure 1. Line Diagram Showing Percentage of Cell viability plotted against the concentration of swasanandam gutika (SG) and DMSO

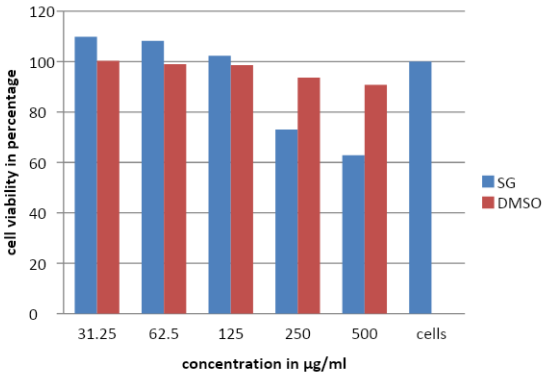

Figure 2. Cell viability Plotted against the concentration of swasanandam gutika and DMSO

DISCUSSION

The *Ayurvedic* multi-ingredient compounds are formulated in a way that the ingredients are capable of counter-balancing toxic effects, if any, present in the herbs or mineral drugs. These particles pass through extensive processing before they are declared fit for internal use. The processes consist of *Shodhan*, *Marana etc.* The herbomineral drugs in ayurveda are claimed to be administered in very low doses, more palatable when compared to herbal dosage forms and also the therapeutic action is faster. Cytotoxicity experiments are a crucial part of a modern pharmaceutical development process. They are a cheap and safe way to get vital information about a new molecule's biological attributes focusing on its basic tolerability. These studies not only save human lives and test animals, but they save the time and resources to be spared on a test molecule

which is a complete failure having no in vitro safety. Indian system of medicine is one of the oldest and well known documented health traditions in the world.

Drug discovery based on traditional information is a key path towards the discovery of new drug. Now a days reverse pharmacology is gaining importance where discovery of leads/formulations is based on the documented clinical experiences and scientific observations through series of studies. Reverse pharmacology based on traditional medicines concentrate on the reversing routine 'laboratory-to-clinic' development to 'clinics-to-laboratories'. Safety is considered as most significant point and the effectiveness becomes a matter of validation. This process is highly useful to find better and safer leads.

The Rasa drugs are facing problems of toxicity issues and hence the safety profiling of these herbomineral preparations is the responsibility of its stake holders. The rasa drugs are administered in the milligram levels and the bioavailability of majority of these drugs were considered very low due to their insoluble nature. It will be difficult to determine the bioavailability of Ayurvedic drugs since they are not a single chemical entity. Thus the concentration of the drug available at the cellular level cannot be predicted.

The present study is taken up an attempt to evaluate the cytotoxic effect of Swasanandam gutika in L-929 mouse fibroblast cells. Here the concentration that might be lethal to 50 percent of the cells is above 500µg/ml. The drug exhibits a trend of cytotoxicity at a concentration above 250µg/ml. It is noticed that the cell viability was increased at lower concentrations of the drug. The increase in cell viability at lower concentrations may be due to the *rasayana guna* of the individual drugs in the formulation. Both *hingula* and *vatsanabha* is having *rasayana guna*. Also the *bhavana dravya* used for preparing pills was *triphala kwatha* which is a unique combination exhibiting the rasayana effect. Thus their combination may exert a synergistic effect thereby increasing the cell viability

CONCLUSION

The results of present study demonstrated that the formulation under study ie Swasanandam gutika, is safe and non-cytotoxic at concentration less than 125µg/ml on mouse fibroblast cells. It is exhibiting cytotoxicity on increasing the concentration of the drug. The cytotoxic effect of the drug at higher doses should be explored by conducting studies in human cancer cell lines. Future research has to be carried out in order to identify the exact mechanism by which the formulation acts in the biological system.

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REFERENCES

1. Frawley D. Ayurvedic Healing - A Comprehensive Guide. Lotus Press: Twins Lake; 2000. [Google Scholar]
2. Kamath SU, Pemiah B, Sekar RK, Krishnaswamy S, Sethuraman S, Krishnan UM. Mercury-based traditional herbo-metallic preparations: A toxicological perspective. Arch Toxicol. 2012;86:831–8. [PubMed] [Google Scholar]
3. Singh SK, Chaudhary A, Rai DK, Rai SB. Preparation and characterization of a mercury based Indian traditional drug: Ras-Sindoor. Indian J Tradit Knowl. 2009;8:346–51. [Google Scholar]
4. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983; 65: 55–63.
5. Van de Loosdrecht AA, Beelen RH, Ossenkoppele GJ, Broekhoven MG, Langenhuijsen MM. A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. J Immunol Methods. 1994; 174: 311-20.

6. Berridge MV, Herst PM, Tan AS. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. Biotechnol Annu Rev. 2005; 11: 127-52.
7. Ramawarrier Kaikulangara, Arogya Kalpa Druma,1st edition, Thrissur :Sahithi Books; 2001;70-85

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