

# Voice Command Browsing (Extension for Google Chrome Browser)

## MINI PROJECT REPORT

*Submitted in partial fulfillment of the requirements for the award of degree*

## BACHELOR OF TECHNOLOGY

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**SCMS SCHOOL OF ENGINEERING AND TECHNOLOGY**

*(Affiliated to M.G. University)*

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ERNAKULAM – 683 582

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**BONAFIDE CERTIFICATE**

This is to certify that the mini project, titled "Voice Command Browsing (Extension for Google Chrome Browser)" by

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submitted in partial fulfilment of the requirement for the award of the degree of Bachelor of Technology, is a bonafide work carried under supervision, during the academic year 2015-2016.

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## **Abstract**

Put Abstract here.....

## **ACKNOWLEDGEMENT**

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# 1 Introduction

## 1.1 OVERVIEW

## 1.2 PROBLEM ANALYSIS

Problem analysis is the process of understanding the actual problems, user needs and proposing solutions to meet those needs. The goal of problem analysis is to gain a better understanding of the problem being solved before development begins. It is the process of gathering and interpreting facts, diagnosing problems and using the information to recommend improvements on the system. Problem analysis is problem solving activities that require intensive communication between users and the system developers. A problem can be defined as the difference between things as perceived and things as desired. The system is studied and analyzed. The system is viewed as a whole and the input to the system are identified. The output from the system is given to various processes.

## 1.3 Scope of study

There is always a preference for antioxidants from natural rather than from synthetic sources, therefore a parallel increase in the methods for estimating the efficiency of such natural antioxidants is an important matter of research. This study aims to evaluate the synergistic action of these drugs when incorporated as a formulation.

The invitro study of various kalpanas of dashamoola would help us explore the mechanism of action of this formulation in the minutest level of the complex cardio vascular system. This could possibly pave the way to access the corresponding systemic action of this formulation and further the estimation of efficacy of the same in Clinical trials.

This study may also help us by throwing light on the aspect as to whether the preparation benefits the patient by acting only at the periphery level enhancing hemodynamic flow or at a central level by acting on cardiomyocytes directly.

The utility of anthracycline antineoplastic agents in the clinic is compromised by the risk of cardiotoxicity. It has been calculated that approximately 10% of patients treated with doxorubicin or its derivatives will develop cardiac complications up to 10 years after the cessation of chemotherapy. This poly herbal combination if found to be cardioprotective may have the potential to reduce the impact of cardiotoxicity when advised as an adjuvant during chemotherapy or can be used as a prophylactic in preventing cardiovascular complications of chemotherapy. It can also become a safe and effective option in peripartum cardiomyopathy.

## 1.4 Objective

- a To evaluate the action of various kalpanas of Dashamoola in doxorubicin induced cardiomyopathy through in vitro cell line study on H9c2 cardiomyoblast cells.
- b Preparation and Physicochemical analysis of various kalpanas of Dashamoola.

## 1.5 Hypothesis

- a *Null hypothesis* : Kalpanas of Dashamoola has no invitro cardioprotective action against doxorubicin induced cardiomyopathy on H9c2 cardiomyoblasts.
- b *Alternative Hypothesis*: Kalpanas of Dashamoola shows invitro cardioprotective activity against doxorubicin induced cardiomyopathy on H9c2 cardiomyoblasts.

## 2 Methodology

### PLAN OF STUDY:

- Review of related literature.
- Pharmaceutical preparation and physicochemical analysis.
- Cardioprotective action by cell line study.
- Data analysis.

### REVIEW OF RELEVANT LITERATURE:

An extensive literary survey of subject will be collected from

- Relevant Ayurveda texts.
- Modern medicine texts.
- Contemporary journals.
- Electronic search (using Pub Med, Google Scholar and Web of Science).
- Other related sources.

**Study Design :** Invitro cell Line Study.

**Study period :** 18 months.

**Study population :** Doxorubicin treated H9c2 cardiomyoblasts

**Sample size :** Not applicable

**Study Setting :**

#### 1 **Drug preparation:**

Department of Rasa sastra & Bhaishajya kalpana  
Govt.Ayurveda College, Tripunithura

#### 2 **Vitro cell line study** in Biogenix Research

Centre, Trivandrum

#### 3 **Analytical study:**

Department of Rasa sastra & Bhaishajya kalpana  
Govt.Ayurveda College, Tripunithura

**Inclusion Criteria :** Not applicable

**Exclusion Criteria :** Not applicable

**Sampling Technique :** Not applicable

**Data Collection :** Primary data collected from lab experiments

### Analytical Study:

#### 1. Organoleptic Characters

- colour
- odour
- taste

## 2. Physicochemical Analysis

- Kashaya and Ksheerapaka
  - pH
  - HPTLC
  - Specific gravity
  - Total ash
  - Acid insoluble ash
- Arka
  - pH
  - Specific gravity
  - HPTLC

### Study Tools:

1. Cytotoxicity assay by MTT method
2. Cytotoxicity assay by Direct microscopic observation.
3. Mitopotential flow cytometry
4. Annexin V/FITC flow cytometry
5. DCFDA Staining

## 2.1 Procedure

### 2.1.1 Cell culture and Treatment

H9C2 (cardiomyoblast cell line) was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecos modified Eagles medium ( Gibco, Invitrogen).

The cell line was cultured in 25 cm<sup>2</sup> tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U $\mu$ g/ml), Streptomycin (100 $\mu$ g/ml), and Amphotericin B (2.5 $\mu$ g/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO<sub>2</sub> incubator (NBS Eppendorf, Germany).

The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

### 2.1.2 Cells seeding in 96 well plate

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100 $\mu$ l cell suspension ( $5 \times 10^4$  cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

### 2.1.3 Dosage Optimization Study

Nontoxic concentrations of various kalpanas of dashamoola will be determined by standard MTT Assay in H9C2 cardiomyoblast cells. Briefly, different concentrations of samples such as 1.75 $\mu$ l, 3.75 $\mu$ l, 6.5 $\mu$ l, 12.5 $\mu$ l, 25 $\mu$ l, 50 $\mu$ l, 100 $\mu$ l volumes of extracts were added to 70% confluent H9C2 cells, incubated for 24 hours and viability was determined by MTT Assay. Morphological changes were recorded using a phase contrast microscopy (Olympus CKX41 20X magnification). LD50 values will be calculated using ED50 plus version 1.0 and sublethal concentrations were used for further studies.



### 2.1.4 Preparation of compound stock

After that the extract solution was filtered through 0.22  $\mu\text{m}$  Millipore syringe filter to ensure the sterility.

Doxorubicin was used to induce toxicity as per methods described by Xiao et al, 2012. After 24 hours the growth medium was removed, Doxorubicin (Sigma Aldrich, US) was added at a final concentration of 0.1% to induce toxicity and incubated for an hour.

Sublethal concentrations of the kalpanas previously filtered through 0.22 $\mu\text{m}$  Millipore syringe filter was added to the 70% confluent cells.

The nontoxic concentrations are to be added in triplicates to respective wells of cells and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

### 2.1.5 Study tools

- **Cytotoxicity Assay by Direct Microscopic observation:**

Entire plate was observed at after 24 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

- **Cytotoxicity Assay by MTT Method:**

The purpose of this assay is to essentially measure the number of metabolically active cells in a 96-well plate.

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30 $\mu\text{l}$  of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator for 4 hours. After the incubation period, the supernatant was removed and 100 $\mu\text{l}$  of MTT Solubilization Solution ( DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 570 nm (*Laura B. Talarico et al., 2004*).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}} \quad (1)$$

- **Mitopotential Flow cytometry:** to check the opening of the mitochondrial permeability transition pore (PTP).
- **APOPTOSIS – Measurement by ANNEXIN V/FITC FLOW CYTOMETRY**  
To assess the rate of apoptosis
- **ROS GENERATION – By DCFDA STAINING**  
To measure the intracellular ROS(Reactive Oxygen Species) production.
- **Method of Preparation – ANNEXURE 1**

## 3 Outcome Variable

Cardioprotective activity in doxorubicin induced cardiomyopathy on H9c2 cardiomyoblasts by MTT method, direct microscopic observation, mitopotential flow cytometry, Annexin V/FITC flow cytometry, DCFDA staining.

## 4 Statistical Analysis

Appropriate statistical technique, if necessary, for the description and summarization of data will be adopted.

## 5 Results and conclusion

The outcome of the work will be concluded after discussion.

## 6 Ethical Consideration

Not Applicable

## 7 Glossary

- **Cardio Protective Agent** - Any protective agent that is able to prevent damage to the heart.
- **H9c2 cardiomyoblast** - A sub clone of the original clonal cell line derived from embryonic rat heart tissue.
- **Cell line** - A cell culture developed from a single cell and having uniform genetic composition. Cell lines provide a pure population of cells which is valuable since it produces a consistent sample and reproducible results.
- **Antioxidants** - A substance (such as beta-carotene or vitamin C) that inhibits oxidation or reactions promoted by oxygen, peroxides, or free radicals.
- **MTT Assay** - It is a colorimetric assay for assessing cell metabolic activity.
- **Mitopotential Flow Cytometry** - An assay to detect the loss of mitochondrial membrane potential that occurs during apoptosis.
- **ROS** - Reactive Oxygen Species is a phrase used to describe a number of reactive molecules and free radicals derived from molecular oxygen.
- **Apoptosis** - Process of programmed cell death that occurs in multicellular organisms.

## ANNEXURE 1

Physico chemical analysis is done and from this the required samples are taken.

### METHOD OF PREPARATION:

#### 1. Dashamoola sritha Kashaya

Roots of the medicinal plant group is crushed and 16 times water is to be added. It is then heated and reduced to 1/8 th . The decoction thus obtained is filtered and used.

#### 2. Dashamoola Ksheerapaka

Roots of the medicinal plant group is crushed and put into a mixture of 8 times milk and 32 times water. It is then boiled and reduced to the amount of milk.

#### 3. Dashamoola Arka

The drugs are cleaned and coarsely powdered. Ten times water should be added to it and is soaked for 24 hrs. Then Arka is extracted by using Arka yantra.

Table 1: Drugs and their Quantity

RAW DRUG	SCIENTIFIC NAME	QUANTITY
Bilwa	Aegle marmelos	1 part
Agnimantha	Premna mucronata	1 part
Shyonaka	Oroxylum indicum	1 part
Gambhari	Gmelina arborea	1 part
Paatala	Stereospermum suaveolens	1 part
Shaalaparni	Desmodium gangeticum	1 part
Prishniparni	Uraria picta	1 part
Gokshura	Tribulus terrestris	1 part
Brihathi	Solanum indicum	1 part
Kantakaari	Solanum xanthocarpum	1 part

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