

# Proliferation Analysis of *Cissus Quadrangularis* and *Cardiospermum Sp.* on MG63 Cell Line

S Geetha, R Vasuki

**Abstract:** Bone strength plays a vital role in prevention and treatment of osteoporosis. While much available therapeutics concentrates on inhibiting the osteoclasts for treatment for osteoporosis, the treatment through stimulation of bone formation is yet to be fully explored. Hence in this study the effect of *Cissus Quadrangularis* and *Cardiospermum halicacabum* and their combination on the proliferation of MG63 cell line was investigated. While the effects of *Cissus Quadrangularis* was found to be adequate to warrant further research and clinical use, that of *Cardiospermum sp.* was found to be better. However the effect of the combination of *Cissus Quadrangularis* and *Cardiospermum sp.* was found to be the most efficient among the three. Hence with extensive validation it can be used as a formulation in pharmaceuticals.

**Index Terms:** Proliferation, Osteoporosis, MG63 Cell Line.

## I. INTRODUCTION

Compromised bone strength increases the risk of fracture. This is an evident characteristic of osteoporosis. It is a skeletal disorder in which the patient suffers from low bone strength. It affects the excellence of life significantly and is related with illness as well as humanity. Hence it is a widespread public health problem. When the population demography consists of a large group of people of advanced age, then osteoporosis can found to be one of the rampant health issues (Bhutani and Gupta, 2013). The formation of osteoblasts and bone resorption by osteoclasts is maintained in a delicate balance for proper functioning. When resorption is more than the formation, it leads to osteoporosis (Lau and Guo, 2011; Roux, 2010). Bone repair is a challenging problem currently faced by the orthopedic surgeons. Osteoporosis can be treated either by inhibiting the osteoclasts or by stimulating bone formation. The current medications available for osteoporosis revolve around inhibiting osteoclasts. A select few therapeutics namely parathyroid hormone analogues such as teriparatide and protect are known to bring about the stimulation of bone formation. However they are expensive when compared to generic medicines such as bisphosphonate which prevent osteoclast mediated bone damage (Hodsman et al., 2005). Despite the cost factor, bone growth stimulation is deemed to an effective approach to cure. Hence there is a

growing stipulate for medicines that stimulate bone formation.

The stimulation of osteoblasts through therapeutics has gained immense importance. Although many mainstream compounds are being tested, the alternative approach of holistic healing through food and traditional medicine is also being explored. Treatment and prevention can be carried out through food and natural therapeutic ingredients or herbal products. This approach if validated can bring about monumental changes in the deterrence and management of osteoporosis (Bakhsh et al., 2013). The MG63 cell line mimics the characteristics of osteoblasts such as creation of osteocalcin and collagen type 1. With its osteoblast like phenotype, the MG-63 cell line assists as a helpful model to check the biological concert of different resources (Lajeuneese et al., 1991; Clover and Gowen, 1994). *Cissus quadrangularis* and *Cardiospermum sp.* are important herbal plants whose natural ability to cure bone disorders has been widely mentioned in the traditional system of medicine. Hence the biocompatibility of both the plants was measured in an in vitro model exhausting the human osteoblast-like MG-63 cell line.

## II. MATERIALS AND METHODOLOGY

### Sample Extraction

Stem and leaves of *Cissus quadrangularis* and *Cardiospermum sp.* were purchased from the market, lapped with sterile purified water to eliminate adherent elements, chopped and air desiccated for a week of time. Then the samples were powdered and stored in air tight container. 25 grams of each sample was weighed and extracted with methanol using soxhlet apparatus for ten hours of time. The extract was then filtered, concentrated using rotary evaporator under reduced pressure. The crude extract was made to a concentration of 100mg/ml and stored for further analysis.

### Cell Culture

Human osteoblast –like MG-63 cell were acquired from NCCS, Pune. The cells were sub cultured and maintained in DMEM media comprising 10% FBS and 1% penicillin-streptomycin (Gibco) at 37°C and 5% CO<sub>2</sub> condition.

### Cell Viability Assay

Cells (1 × 10<sup>5</sup>/well) were plated in 24-well plates and nurtured in 37°C with 5% CO<sub>2</sub> complaint. After the cell spreads the convergence, the numerous meditations of the examples (*Cissus quadrangularis* alone, *Cardiospermum sp.* alone, Mixture of both extracts) were new and incubated for 24hrs, 48 hrs and 72 hrs. Cells with DMEM alone (control) was also maintained.

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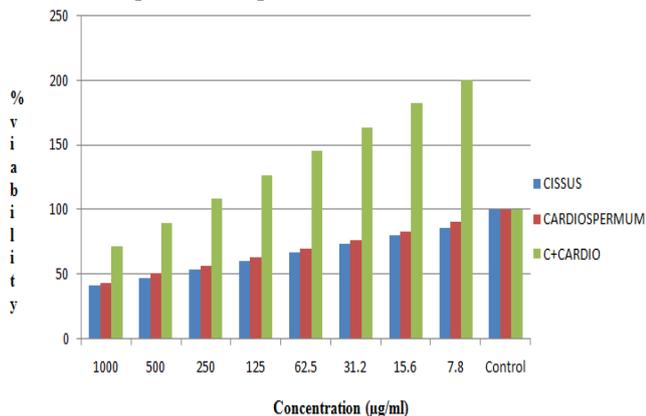
## Proliferation Analysis of *Cissus Quadrangularis* and *Cardiospermum Sp.* on MG63 Cell Line

Subsequent to incubation, the samples were separated from the well and lapped by phosphate-buffered saline (pH 7.4). 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) was used as well as incubated for 4 hours. Subsequent to incubation, 1ml of DMSO is added in all the wells. The absorbance at 570nm is calculated with UV- Spectrophotometer utilizing DMSO as the empty (Mosmann,1983). The % cell feasibility was considered utilizing the subsequent formula:

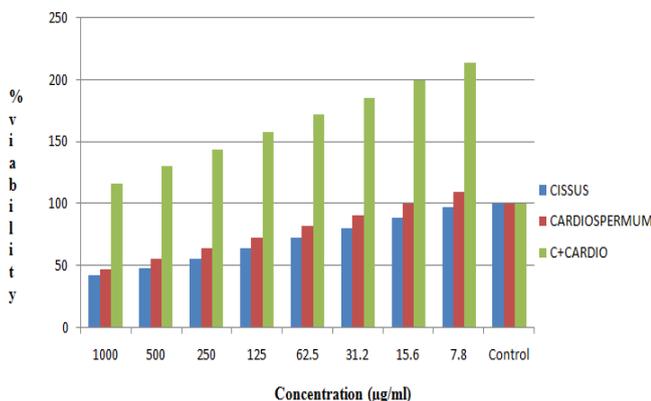
% Cell viability =  $A_{570}$  of treated cells /  $A_{570}$  of control cells × 100

### III. RESULTS AND DISCUSSION

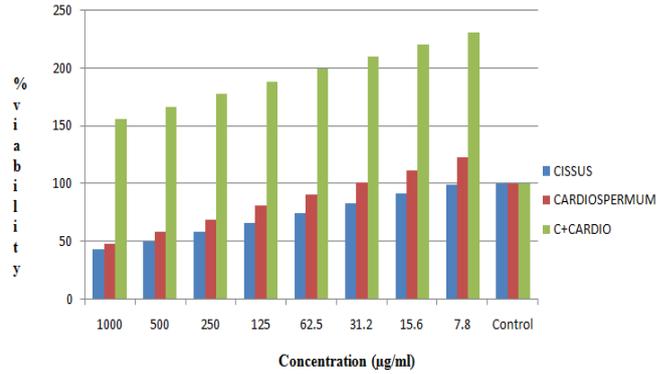
The influence of samples on the osteoblast proliferation was explored for 24 hrs, 48 hrs and 72 hrs of time with varied concentrations of the samples. The results clearly revealed that the samples did not exert any cytotoxicity towards the cell line. The proliferation was increased with increasing incubation time. The effect of *Cissus quadrangularis* and *Cardiospermum sp.* on the proliferation of osteoblast cell line was found to be more or less similar but the combination of both the extracts showed profound increase on the proliferation. The results clearly exhibited the stimulatory effect of samples on the proliferation.



**Fig.1 Proliferation activity of samples on MG63cell line for 24Hrs**



**Fig.2 Proliferation activity of samples on MG63cell line for 48Hrs**



**Fig.3 Proliferation activity of samples on MG63cell line for 72Hrs**

The stimulatory effect of Water Cress Rutin on the MG-63 cell proliferation was observed in a similar study by Hanbit hyun *et al.*, (2014). All verified attentions from 1 - 50 µg/ml showed positive results in the study where the Watercress Comprising Rutin and Rutin unaided were studied for their effect on the Proliferation as well as Osteogenic. Discrimination of Human Osteoblast akin to MG-63 Cells. Similar positive results were obtained in this study.

Sridhar Muthusami *et al.*, (2011) studied the Effects of *Cissus quadrangularis* on the Proliferation, Difference and Matrix Mineralization of Human Osteoblast like SaOS-2 Cells and exposed that *C. quadrangularis* conduct has amplified the DNA synthesis of human osteoblastic SaOS-2 cells representing amplified propagation of these cells.

Jianhong Yang *et al.*, (2010) studied that Insulin arouses osteoblast proliferation and difference through ERK and PI3K in MG-63 cells and reported that Insulin enthused the propagation of MG-63 cells in a time- and dose-dependent manner.

### IV. CONCLUSION

The results of this research work were found to be in congruence with similar studies that showed the significant effect of herbal plants like *Cissus quadrangularis* on the proliferation of osteoblastic SaOS-2. In this study the unique combination of *Cissus quadrangularis* and *Cardiospermum sp.* were found to be efficient in increasing the proliferation of MG63 cells. Thereby supporting the bone cell growth and metabolism.

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