ORIGINAL RESEARCH PAPER

Pharmacology

SCREENING OF AYURVEDIC HERBS FOR ITS OSTEOBLAST PROLIFERATION ACTIVITY USING MG 63 CELL LINE

KEY WORDS: Osteoporosis, Ayurveda, MG 63 cell line.

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ABSTRACT

Bone mineral density is decrease in osteoporosis, which may leads to prominent fracture with trauma and severe stress to bone and affect quality of life significantly. Many Ayurveda herbs are mentioned and consumed in India for treatment of bone fracture and related disorder. However, no systematic and scientific study has been done to validate their use for their effect on bone. So, aim of the current study is to screen Ayurvedic herbal medicine for their effect on the osteoblast cells proliferation, which is major bone forming cell. To achieve this aim we selected four drugs (bark of *Terminalia arjuna*(Ta), rhizomes of *Curculigo orchioids*(Co), stem of *Tinospora cordifolia*(Tc), and *Cissus quadrangularis*(Cq)) on the basis of thorough literature review. Aqueous and ethanolic extract of the selected plant were prepared, screened for their cell proliferation activity in MG 63 cell line using MTT assay.

INTRODUCTION:

Bone is developed through the activities of endochondral and intramembranous ossification. Bone necessity to be continuously replaced to maintain its strength and integrity. Bone remodeling is systematized by two opposing activities; like bone formation by osteoblasts, which create the organic bone matrix and increase the thickness of the bone, and bone resorption which is done by osteoclasts, which soften bone mineral and extracellular matrix and cause bone thinning[1]. Osteoporosis causes progressive bone loss, which renders the bones susceptible to fractures and is popularly known as "the silent disease" because there is no early sign of diseases[2]. These fractures are common and have extensive medical and personal burden on individualsand also on the nation. Osteoporosis can be prevented, diagnosed and treated before any fracture occurs and effective treatments can be used to decrease the risk of further fractures even after first fracture has been occurred [3].

Herbal Plants have been the rich source of medicines throughout the world from thousands of years and still provide new remedies to mankind. Natural products for the management of osteoporosis are largely phytoestrogens, which include isoflavones, lignins, flavonoids, and coumestans that share structural and functional similarities with naturally occurring or synthetic estrogens [4]. Due to the probable and actual side effects of the allopathic agents, currently the usage of the Ayurveda agents is significantly increased. Moreover, these agents are more safe and can be used for the prolong condition also. On the basis of literature review we selected four herbal drugs ((bark of *Terminalia arjuna* (Ta) [5] , rhizomes of *Curculigo orchioids* (Co) [6], stem of *Tinospora cordifolia* (Tc) [7], and *Cissus quadrangularis* (Cq) [8]) and prepared aqueous and ethanolic extracts of it to study the pharmacological activity on the MG 63 cell line using a standard cell proliferation MTT assay.

MATERIALS AND METHODS:

Collection and authentication of selected plants

Stem of *Tinospora cordifolia* and *Cissus quadrangularis* were collected from Pethapur, region of Gandhinagar, Gujarat while bark *Terminalia arjuna* and rhizomes of *Curculigo orchioides* were procured from LalluVrajlal Gandhi, Ahmedabad, Gujarat. Collected plants were authenticated by its microscopy and morphology and compared with standard reference and herbarium sheets were submitted to Department of Pharmacognosy, K. B. Institute of Pharmaceutical Educationand Research, Gandhinagar, Gujarat, India. Collected plant material washed and dried under shadow and powdered.

Preparation of extracts for osteoblast proliferation assay

Aqueous and ethanolic extract of each plant was prepared by maceration for 48 hours. For preparation of aqueous extract 100g of powdered drug twice extracted with 1000ml of water and boiled on waterbath for 6 hours and allow to stand for overnight. Filtrate of both extraction steps was pulled together and concentrated using rota evaporator and % yield of each extract was calculated(% Yield: TaA (18.3 %), TcA (13 %), CoA(19.7 %), CqA (20.3 %)).Ethanolic extract prepared by twice refluxing with ethanol on waterbath for 6 hours at 55°C.Filtre from both extraction steps combined and solventremoved by rota evaporator(% Yield: TaE (20.6 %), TcE (6.6 %), CoE (12.2 %), CqE (5 %)). Each extracts were properly labeled and stored in freeze until use for screening. All the extracts were screened for the presence of the phytoconstituents.

Procurement of MG 63 cell line and it's Maintenance [7]

MG-63 cell line was used for the screening of extracts for its cell proliferation activity. MG-63 was the human bone osteosarcoma cell line which was procured from NCCS, Pune. According to ATCC protocol cell line was allow to grow in minimum essential medium-Eagle supplemented with 1X antibiotic antimycotic solution (A007, Himedia, India) and 10% fetal bovine serum(FBS-RM1112, Himedia, India) under standard growth conditions (temperature 37 C, 5% CO_2 and 95% humidity)in a CO_2 incubator (Forma Scientific, USA). After confluent monolayer cells were trypsinized with 0.25% trypsin–0.2%EDTA in Dulbecco's phosphate buffered saline (T-001, Himedia, India) and subcultured to obtain enough number of cells for proliferation assay. For the proper growth of the cell media was changed every alternate day. After achieving about 90% confluency, the cells were seeded on to 96well microtitre plates for cell proliferation assays. All the chemicals and reagents utilized in this investigation were analytical grade.

Cell viability Assay

Prepare a cell suspension after trypsinization in a fixed volume of cells. Take 50uL of cell suspension and blend it with an equivalent volume of trypan blue. Mix it gently and load on haemocytometer for cell count. Living cells were as clear form and while non-living cells with trypan blue dye appear a blue colored under inverted microscope. Calculate the number of cells/ ml, and the total number of cells, using the standard formula.

MTT cell proliferation assay [9]

Cell dilution was prepared accordingly to plate 10000 cells/ well in 96 well plate. Cells were allow to adhere to the bottom of well for 24 hour at 37°C and in 5% CO_2. After complete adherence cells were treated with plant extracts at 3 different concentrations (1000µg/ml, 100µg/ml and 10µg/ml) and allow proliferating for 48 hours. Alendronate (ALD) was used as a positive control of cell proliferation assay. Drug treatment was followed by addition of

20µl of MTT reagent (0.5 mg/ml) to each well and cells were incubated for 4 h at 37°C in dark for formation of purple colored formazan crystals. All media and MTT reagent was pipette out precisely without disturbing the cell layer at the bottom of well and 200 µl DMSO was added to each well to dissolve the formazan crystals. Using a microplate reader, the optical density (OD) was recorded at 570 nm. Percentage of cell proliferation activity was determined by using standard formula. The Data is statically analyzed by applying one-way analysis of variance (ANOVA) followed by Tukey Test, using Graph Pad Prism software. The data is expressed as mean ± standard error of the mean (SEM). The results were considered statistically significant if the $P < 0.05$.

RESULTS:

Total viable cells and % cell viability of cell line was calculated by trypan blue assay and it was found $2.28*10^6$ cells/ ml and 92.81% respectively which suitable to perform proliferation study. Cell proliferation activity of aqueous and ethanolic extracts of selected plants was screened by MTT assay using MG 63 cell line shown in figure 1 and 2. Alendronate as standard, increase proliferation of MG 63 cells as compare to cells grown in basal media(BM) in dose dependent manner but in lower concentration negligible changes shown.

Bark of Ta was used for the bone aliment of post-menopausal osteoporosis from the time of decades. In current assay, TaA shows markely cell proliferation in the higher concentration as compare to lower concentrations. While TaE shows significant cell proliferation at both 1000µg/ml and 100µg/ml concentration. At lower concentration 10µg/ml also show notable stimulation to cell. So, both the extracts shows dose dependent action.

Stem of Tc (Guduchi) mentioned as a remedy of rheumatoid arthritis and inflammatory diseases in ancient Indian Ayurvedic literature. According to current data TcA shows significant cellproliferation of at higher concentration 1000 µg/ml only and TcE did not show a remarkable effect on cells.

Rhizomes of Co (Kali mushali) is wildely used as an anti-inflammatory drug. In our study, higher concentration of CoA and CoE has decreased the effect on cell proliferation. While other two concentrations having significant proliferation activity on MG 63 cell line.

Stem of Cq (Hodjod) was widely used for the fracture and bone disease in india. CqA and CqE at higher concentration show maximum osteoblast proliferation activity while other two lower concentrations of both the extracts have not any notable proliferation.

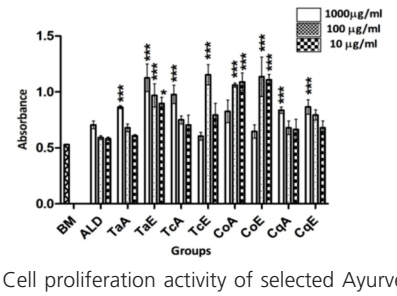

Figure. 1: Cell proliferation activity of selected Ayurveda plants. BM- Basal media. All values represent mean ± S.E.M; n=3; ***p < 0.001, *p<0.05 compared with BM.

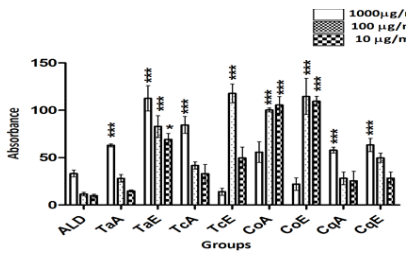

Figure. 2: Percentage proliferation of selected Ayurveda plants. All values represent mean ± S.E.M; n=3; ***p < 0.001, *p<0.05 compared with BM.

DISCUSSION:

Currently, the prevalence of osteoporosis among women in India become a major concern across the country, estimates suggest that of the 230 million Indians expected to be over the age of 50 years in 2015, 20%, ie, ~46 million, are women with osteoporosis [10]. The available therapeutic agents are associated with certain intolerable adverse effects, which arise the question for its chronic use [11]. Certain plant compounds, which have been characterized as phytoestrogens, have shown a weak estrogenic effect on bone in human and animal studies. It is also reported that [5]. Some herbal drugs have been traditionally used in Ayurveda to accelerate the healing of bone fractures and to strengthen the bones.

In our study, our selected herbal drugs extracts, shows proliferation of MG 63 osteoblast cells, which will justify the use of these plants for the fractures and osteoporosis. Osteoblast proliferation is a one of the mechanism to improve bone health of these drugs and which will certain the use of it. Ethanolic extracts of *Terminalia arjuna* show significant cell proliferation activity in all concentrations in dose dependent manner. While stem of *Cissus quadrangularis* also show dose dependent cell proliferation activity but there is not as markable as *Terminalia arjuna.*

CONCLUSION:

The above data represent that bark of *Terminalia arjuna* and stem of *Cissus quadrangularis* show dose dependent cell proliferation activity on MG 63 osteoblast like cell line. Above study indicates that, the extracts might have osteoblast proliferation activity. So cell proliferation of these plants has to be studied for their various biological activities. And these drugs can be use for the preparation of herbal formulation for the treatment of osteoporosis.

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