Original Article

Mg-63 Cell Line Response on Phytosterol Treatment Along with Pharmacokinetic Assessment of Phytosterol Effectiveness in Osteoarthritis Using Insilico Methods

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Abstract

Objective- Osteoarthritis is a heterogenous disease affecting synovial joints of hands, knees, hips and spine and is characterized by progressive degeneration of articular cartilage with a progressive episodic synovitis and bone remodeling. Phytosterols are plant based cholesterol which have proven to show numerous therapeutic benefits. Therefore this study was conducted to determine the anti-osteoarthritis effect of phytosterols using MG-63 cell line for *in-vitro* analysis as well as *in-silico* methods were being used.

Methodology- *In-silico* and *in-vitro* studies were conducted using computational methods to predict the pharmacokinetic parameters and biological activity of the phytosterols. MG 63 cell line was used in order to assess their response toward phytosterol treatment. Online servers such as pkCSM, OSIRIS, Molinspiration, Swiss ADME were being used in order to assess the physiochemical, pharmacokinetic and pharmacological property of the compound.

Result- The *in-silico* and *in-vitro* methods used for analysis determined a wide range of results which provides enough evidence regarding anti-osteoarthritic effect of phytosterols. Stigmasterol has shown anti-eczematic, lipid peroxidase inhibition, immunosuppression, nootropic and bone disease treatment activity whereas β-sitosterol has shown chemoprotective, hepatoprotective, lipid peroxidase inhibition and bone disease treatment activity.

Conclusion- Therefore this study provides evidence which shows light on effectiveness of phytosterol for management of osteoarthritis and also towards the response of MG-63 cell line toward phytosterol treatment and this could be utilized for further studies related to the topic

Keywords- Osteoarthritis, phytosterols, Swiss ADME, Molinspiration, stigmasterol, β-sitosterol, synovial joint, physiochemical

1) INTRODUCTION:

With increase in the lifespan of human beings all throughout the world, prevalence of diseases has also increased, of which arthritis is one of the most common disorder affecting large population of humans irrespective of their age, races and sexes. Arthritis is a degenerative disorder of the joints and bones with over 100 types of which osteoarthritis, rheumatoid arthritis, psoriatic arthritis and inflammatory arthritis have most prevalence(1). Osteoarthritis (OA) is a heterogeneous disease affecting synovial joints of hands, knees, hips and spine. It is characterized by progressive degeneration of articular cartilage with progressive episodic synovitis and bone remodeling(2). Modern medical research has shown that OA develops after articular cartilage injury and chondrocyte death accompanied by cartilaginous ossification and subchondral bone hyperplasia. Several risk factors associated with OA including genetic predisposition, ageing, obesity and joint mal-alignment have been put forward but the actual pathogenesis of OA yet remains unclear(3). Treatment of OA involves alleviation of pain , reducing stiffness, improving quality of life and restoring fuctional capacities. Management of OA includes weight loss, low impact aerobic exercise, acupuncture, glucosamine, chondroitin sulphate therapy and surgery.
Phytosterols , plant based sterols , having chemical and biological similarity to functions of cholesterol but with a slight moderation have shown immense therapeutic effect against cardiovascular disease as well as protective effect against various chronic ailments, diabetes, cancer, hepatoprotective(4).Phytosterols are byproducts of isoprenoid biosynthesis pathway produced from acetyl coenzyme A via squalene. Campesterol, stigmasterol and β-sitosterol are the most commonly occurring phytosterols obtained from wide variety of plant based foods such as nuts, seeds, vegetable oils, cereals and legumes.(5)(6)

Stigmasterol available as unsaturated phytosterol has shown tremendous potential in reducing cardiovascular disease and exerts anti-angiogenic and anti-cancer effect by downregulation of tissue necrotic factor-alpha and VEGFR-2. β-sitosterol

is a bioactive phytosterol present in plant cell membrane, accounts for about 65% of human herbal nutrition formation has been reported to posses anxiolytic, analgesic, immunomodulatory, antimicrobial, anticancer ,lipid lowering and hepatoprotective properties in many scientific reports(5).
MG-63 cell lines are human osteoblastic cell lines which have been used in many scientific research to determine the action of various drugs or compounds. Metabolic profiling of cultured cells on exposure to drug molecule helps to measure cellular metabolic response and also offers an insight about drug metabolism at cellular level and also helps to determine the drug efficacy as well as toxicity. (7)
Various studies conducted have shown that phytosterol like stigmasterol and β-sitosterol has potential therapeutic benefits but there wasn't much evidence regarding anti-osteoarthritic effect. Therefore this study was conducted to study about the anti-osteoarthritic effect of the phytosterols by using *in-silico* as well as *in-vitro* methods. The pharmacokinetic parameters, potential adverse effects, toxicity studies, biological activity and structural activity relationship of the compounds were analyzed and summarized. Various online servers and web resources were employed in this study. The use of offline and online tools for prediction and evaluation of various properties and parameters of the drug have shown light to evidential conclusion. The use of *in- silico* method for assessing the pharmacology of the drug candidate helped to understand the absorption, distribution, metabolism, excretion and toxicity properties and also helped in optimization of novel molecules affinity to the target site. The evidence provided in our study will help future researches conduct further studies related to this topic.

2) METHODOLOGY:

A strategic approach was designed and implemented in order to asses the physiochemical properties, pharmacokinetic properties and pharmacological activity of stigmasterol and β- sitosterol and to determine the significance of phytosterol treatment for management of OA. Various *in-silico* tools were being used for analyzing the compound and its properties. *In-vitro* methods were also used for determining the biological activity of the compound.

2.1 Estimation of pharmacokinetic profile:

The canonical smiles of Stigmasterol and β-Sitosterol were taken from the PubChem online server. The pharmacokinetic studies were performed using pkCSM and Swiss ADME (8)(9).The ADMET profile is taken from the above-mentioned online server by using the canonical smiles. The pkCSM and Swiss ADME gives the absorption, distribution, metabolism, excretion, and toxicity profile of the drug. pkCSM online tool is used to study pharmacokinetic properties of the drugs and is found to be reliable. Evaluation of phytosterols for its physiochemical properties, pharmacokinetic properties, pharmacokinetic properties to study the Osteoarthritis activity used the pkCSM tool(8)(10).

2.2 In silico prediction of toxicity

The toxicological screening plays a major role in the withdrawal of the drug in order to prevent damage or failure of the organ system and other complications. To know the toxicity profile OSIRIS® property explorer program where the chemical structure of the compounds was referred from PubChem. Tumorigenicity, mutagenicity, irritant, reproductive effect, drug-likeness are measured using a colour scale (11). TPSA, drug-likeness, overall drug score can also be generated. The compound 3-Aminothiophene-2-Acylhydrazones was tested for nontoxic, analgesic and anti-inflammatory lead candidates. Phytosterol were studied using OSIRIS property explorer. The insilico tool showed good drug score and lower toxicity (11)(12).

- Green: Safe to use
- Yellow: medium risk of toxicity
- Red: high risk of toxicity

2.3 Molecular Property Assessment:

In silico screening of the selected drugs were performed using MOLINSPIRATION® software to evaluate the drug likeness and to predict their bioactivity (12). It also helps to screen the fragments present in the drugs. The drugs with a score of 0.00 for bioactivity is considered to be ideal biological activity, bioactivity score ranging from -0.50 - 0.00 indicates moderate activity and <0.00 means, the drug is inactive.

2.4 Prediction of biological activity of Stigmasterol and β-Sitosterol using PASS online server:

The PASS (Prediction of Activity Spectra for Substances) online web resource was used to predict the biological activity of the drugs. The biological activity spectrum of the drugs is predicted on the basis of the structural formula of the drugs. The analysis is purely based on the structure- activity relationship (13). The biological activities result from interaction of the compounds present in the drugs and the biological objects were analyzed using PASS online server. The activity of drug is dependent upon its activity and therefore SAR of the drug should be estimated for its pharmacological effects (12). The adverse effects of the drugs were also predicted using PASS online web resource. PASS online software is used to assess the biologically active compounds. The software can predict more than 300 pharmacological effects and biochemical mechanism. The prediction is on the basis of the structural formula of the natural substance. This may help us in finding new targets of the drug that is tested (12).

2.5 In-vitro cell line studies:

2.5.1 Alkaline Phosphatase Assay

Preparation of cell culture:

MG-63 (Human osteoblastic cells) cell line was purchased from the National center for cellular sciences, Pune, and cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 u/ml penicillin and 100 µg/ml streptomycin and maintained under an atmosphere of 5% CO_2 at 37ºC.

Procedure:

The measurement of alkaline phosphatase activity in the Stigmasterol and β-Sitosterol treated cell culture supernatant was carried out using an alkaline phosphatase assay kit, as per the manufacturer's instruction (Sigma, USA). 980µl of reaction buffer was transferred into one cuvette (blank) and then 960 µl of reaction buffer was pipetted into additional cuvettes (one for each test and enzyme control). 20 µl of 0.67M pNPP (p-nitrophenylphosphate) solution was added to each cuvette (blank, test and control)..The test sample 20 µl was added to test cuvette. And then 20 µl of the diluted alkaline phosphatase solution was added to the enzyme control. All the solutions were immediately mixed by inversion and the cuvettes were equilibrated at 37°C. After incubation the Optical Density was immediately measeured at 405 nm using spectrophotometer for 5 minutes. The maximum linear rate (ΔA405nm/minute) were obtained for the test, blank and control.

Units/mL= (ΔA405nm/min Test – ΔA405nm/min Blank) (df) (VF)

_____

(18.5) (VE)

df = Dilution Factor (100)

VF = Volume (in ml) of assay (2 ml)

18.5 = Millimolar extinction coefficient of pNPP at 405 nm

VE = Volume (in ml) of sample solution used (0.02 ml)

2.5.2 Wound healing Assay

Cell culture:

MG-63 (Human osteoblastic cell) cell line was purchased from the National center for cellular sciences, Pune, and cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 u/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37ºC.

Cells treatment:

MG-63 cells were seeded into a six-well tissue culture dish and allowed to grow to 90% confluency in a complete medium. The cells were treated with 200 µg/ml of Stigmasterol and β-Sitosterol sample and incubated for 24 h.

Wound healing assay:

A wound-healing assay was used to assess cell migration of both cancer and non-cancer cell lines upon treatments. MG-63 cells were seeded into a six-well tissue culture dish and allowed to grow to 90% confluency in a complete medium. Cell monolayers were wounded by a plastic tip (1 mm) that touched the plate as described (Xu and Deng, 2006). Wounded monolayers were then washed four times with a medium to remove cell debris and incubated in 1 % FBS medium. The cells were treated with 100 µg/ml of Stigmasterol and β-Sitosterol sample and incubated for 24 h. Cells were monitored under an inverted microscope equipped with a camera. The wound area was measured using Image-J software (NIH, Bethesda, MD, USA). The wound area percentage was calculated as the wound area of control and treated sample.

3) RESULT:

3.1 Pharmacokinetic profile evaluation:

The ADMET parameters of Stigmasterol and β-Sitosterol were analyzed using pkCSM online server. The results generated from pkCSM server are given in table S1.

Stigmasterol and β-Sitosterol shows slight solubility in aqueous solvents which eventually affects the distribution and absorption of the compound. The normal range of solubility is given in table S8. The normal range of Caco2 permeability is given in table S9. Stigmasterol and β-Sitosterol shows very low Caco2 permeability. However, it shows low intestinal absorption. The normal range of intestinal absorption is mentioned in table S10. The Blood Brain Barrier permeability of Stigmasterol and β-Sitosterol was found to be -0.544 & -0.082 (log BB). Meanwhile its CNS permeability was found to be -1.448 &-1.243 (log PS). Stigmasterol and β-Sitosterol is a CYP3A4 substrate and has a total clearance of -0.043 & -0.034ml/min/kg respectively.

Stigmasterol and β-Sitosterol does not show AMES toxicity and is not a renal OCT2 substrate. The maximum tolerated human dose of Stigmasterol and β-Sitosterol is 0.034 & 0.0224 mg/kg/day in accordance with the results obtained from pkCSM online server. The LD50 value of Stigmasterol and β-Sitosterol was found to be 2.227 & 1.125 mol/kg. The compound does not show hepatotoxicity or skin sensitization. The bioactivity score of the compound Stigmasterol and β-Sitosterol were studied using MOLINSPIRATION. The use of MOLINSPIRATION showed promising bioactivity score for drug targets and the study suggests that this pharmacokinetic tool is reliable for performing computational drug designing and pharmacokinetic analysis (9,14) .

3.2 Assessment of pharmacokinetic profile using Swiss ADME:

The physiochemical properties of Stigmasterol and β-Sitosterol determined from Swiss ADME have been listed in table S2. The lipophilicity profile of Stigmasterol and β-Sitosterol is given in table S3. The pharmacokinetic profile of Stigmasterol and β-Sitosterol obtained from Swiss ADME implies that the compound has high GI absorption. The water solubility profile of stigmasterol and β-sitosterol is mentioned in table S4. The result obtained from Swiss ADME also indicated that both the compounds are p- glycoprotein substrate (15,16).

3.3 Prediction of toxicity and Drug likeness using OSIRIS property explorer:

The physiochemical properties of the compound were determined using OSIRIS property explorer and is given in table S6. The compound did not show any indication of mutagenicity, tumorigenicity, reproductive effect or irritancy. The OSIRIS property explorer indicated green for all the above-mentioned parameters adding to its safety profile. The TPSA of a compound ultimately determines the absorption of the drug. It also contributes to the transportation and penetration of the drug(17). The TPSA of the compound was observed to be 20.23 $Å^2$ from the OSIRIS property explorer which indicates good permeability of the compound.

The summation of the molecular weight of a compound, its toxicity risks and C Log P is known as the drug score. The drug score is essential as it aids in evaluating the capability of a chemical component to meet demands of a possible drug. The drug score of Stigmasterol and β-Sitosterol was found to be 0.25 &0.40 respectively.

3.4 Bioactivity score prediction using MOLINSPIRATION®:

The bioactivity score of the drug was estimated for drug binding to nuclear receptor ligand, protease inhibitor, enzyme inhibitor, G- protein coupled receptor, ligand kinase inhibitor and ion channel modulator using Molinspiration. The bioactivity scores more than 0.00 indicates biological activity, scores ranging from -0.5 to 0.00 indicates moderate activity and a score less than 0.00 is considered inactive(18). The results obtained from Molinspiration are enlisted in table S5.

3.5 Evaluation of bioactivity of Stigmasterol and β-Sitosterol using PASS online:

On evaluation of biological activity using PASS online server, Stigmasterol was found to posses anti-eczematic, bone disease treatment, lipid peroxidase inhibitor, immunosuppressant and nootropic activity and β-sitosterol was found to posses chemoprotective, hepatoprotective, lipid peroxidase inhibitor, immunosuppressant and bone disease treatment properties. The result obtained from PASS online server has been mentioned in table S7a for Stigmasterol and table S7b for β-Sitosterol.

3.6 Alkaline phosphatase assay:

After incubation of the test and control the alkaline phosphatase (ALP) concentration and the optical density (OD) produced in each was being measured. The test solution showed an increase in ALP concentration with the time duration and the control showed high concentration due to direct addition of ALP to the solution. The optical density measured at 405 nm using spectrophotometer indicated an increase in OD of the test solution with increase in time whereas the control showed high OD due to high concentration of ALP in the solution and the blank showed least OD due to absence of ALP. The ALP activity has been recorded in table S11 and the OD value obtained at 405nm is shown in table S12.

3.7 Wound healing assay:

On treatment of the cell plates with stigmasterol and sitosterol a prominent effect of wound healing was seen. The migration of the cells and the wounding healing in test as well as control toward Stigmasterol treatment is demonstrated in figure 1 and the response towards β-Sitosterol treatment is demonstrated in figure 2.

Table S1: Pharmacokinetic profile of Stigmasterol & β-Sitosterol

Parameter	Stigmasterol Predicted Value	β-Sitosterol Predicted Value
Water solubility (log mol/L)	**-7.46**	**-7.90**
Intestinal absorption (human) (% Absorbed)	**Low**	**Low**
P-glycoprotein I/II inhibitor	**No**	**No**
VDss (human) (log L/kg)	**-1.217**	**-1.621**
Fraction unbound (human) (Fu)	**0.065**	**0.825**
BBB permeability (log BB)	**-0.544**	**-0.082**
CNS permeability (log PS)	**-1.448**	**-1.243**
CYP2D6 substrate	**No**	**No**
CYP3A4 substrate	**Yes**	**Yes**
CYP1A2/2C19/2C9/ 2D6/3A4 inhibitor	**No**	**No**
Total Clearance (log ml/min/kg)	**-0.043**	**-0.034**
Max. tolerated dose (human) (log mg/kg/day)	**0.034**	**0.025**
hERG I/II inhibitor	**No**	**No**
Oral Rat Acute Toxicity (LD50) (mol/kg)	**2.227**	**1.125**
Oral Rat Chronic Toxicity (LOAEL) (log mg/kg/day)	**1.808**	**1.564**

Table S2. Physicochemical properties of Stigmasterol& β-Sitosterol using Swiss ADME

Parameter	Stigmasterol Value	β-Sitosterol Value
Molecular weight	414.71 g/mol	414.71 g/mol
Num. heavy atoms	30	30
Num. arom. heavy atoms	0	0
Fraction Csp3	0.86	0.93
Num. rotatable bonds	5	6
Num. H-bond acceptors	1	1
Num. H-bond donors	1	1
Molar Refractivity	132.75	133.23
TPSA	20.23 Å²	20.23 Å²

Table S3: Lipophilicity profile of Stigmasterol & β-Sitosterol profile using Swiss ADME

Lipophilicity		
Parameter	**Stigmasterol Value**	**β-Sitosterol Value**
Log $P_{o/w}$ (iLOGP)	5.01	4.79
Log $P_{o/w}$ (XLOGP3)	8.56	9.34
Log $P_{o/w}$ (WLOGP)	7.8	8.02
Log $P_{o/w}$ (MLOGP)	6.62	6.73
Log $P_{o/w}$ (SILICOS-IT)	6.86	7.04
Consensus Log $P_{o/w}$	6.97	7.19

Table S4: Water solubility profile of Stigmasterol & β-Sitosterol using Swiss ADME

Water Solubility		
Parameter	**Stigmasterol Value**	**β-Sitosterol Value**
Log *S* (ESOL)	-7.46	-7.9
Solubility	1.43E-05 mg/ml; 3.46Ee-08 mol/l	5.23E-06 mg/ml;1.26E-08mol/l
Class	Poorly soluble	Poorly soluble
Log *S* (Ali)	-8.86	-9.67
Solubility	5.71E-07 mg/ml; 1.38E-09 mol/l	8.90E-08 mg/ml;2.15E-10mol/l
Class	Poorly soluble	Poorly soluble
Log *S* (SILICOS-IT)	-5.47	-6.19
Solubility	1.40E-03 mg/ml; 3.39E-06 mol/l	2.69E-04 mg/ml;6.49E-07mol/l
Class	Moderately soluble	Poorly soluble

Table S5. Bioactivity score of Stigmasterol & β-Sitosterol using MOLINSPIRATION®

Parameter	Stigmasterol Value	β-Sitosterol Value
GPCR Ligand	0.12	0.14
Ion channel modulator	-0.08	0.04
Kinase inhibitor	-0.48	-0.51
Nuclear receptor Ligand	0.74	0.73
Protease inhibitor	-0.02	0.07
Enzyme inhibitor	0.53	0.51

Table S6. Toxicity profile of Stigmasterol & β-Sitosterol using OSIRIS property explorer

PARAMETERS	Stigmasterol (scores)	β-Sitosterol (scores)
Mutagenic	**GREEN**	**GREEN**
Tumorigenic	**GREEN**	**GREEN**
Irritant	**GREEN**	**GREEN**
Reproductive effect	**GREEN**	**GREEN**
TPSA	20.23	20.23
Drug likeness	-4.00	1
Drug score	0.25	0.40

Table S7a. Evaluation biological activity of Stigmasterol using PASS online tool

Pa	Pi	Activity
0.806	0.017	Anti-eczematic
0.706	0.005	Bone diseases treatment
0.779	0.004	Lipid peroxidase inhibitor
0.782	0.007	Immunosuppressant
0.702	0.041	Nootropic

Table S7b. Evaluation biological activity of β-Sitosterol using PASS online tool

Pa	Pi	Activity
0.831	0.003	Chemopreventive
0.815	0.004	Hepatoprotectant
0.779	0.004	Lipid peroxidase inhibitor
0.762	0.009	Immunosuppressant
0.718	0.005	Bone diseases treatment

Table S8. Range of water solubility

Value of Water Solubility (Log S)	Solubility
More than 0	Highly soluble
0 to -2	Moderately soluble
-2 to -4	Slightly soluble
Less than -4	Insoluble

Table S9. Range of Caco2 permeability value

Caco2 assay value	Range	Percentage
Papp ≤ 10-6 cm/s	Low	0-20%
10-6cm/s < Papp ≤ 10 x 10-6 cm/s	Medium	20-70%
Papp > 10 x 10-6 cm/s	High	70-100%

Table S10. Range of human intestinal absorption in percentage

HIA in percentage	Absorption rate
100-67	High
66-33	Moderate
32-0	Low

Table S11. ALP activity (Units/ml)

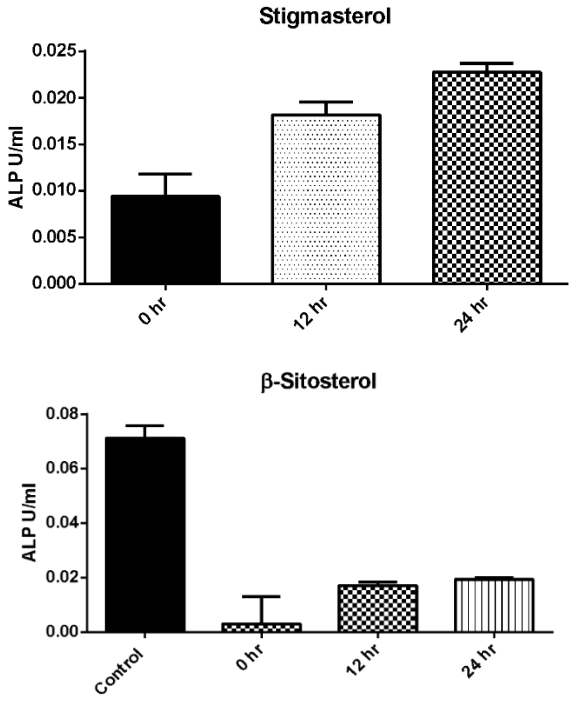
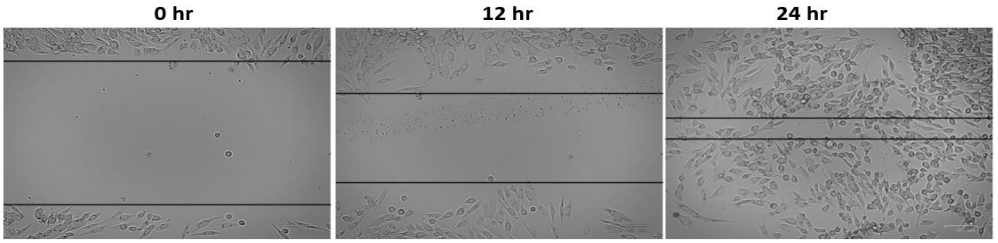
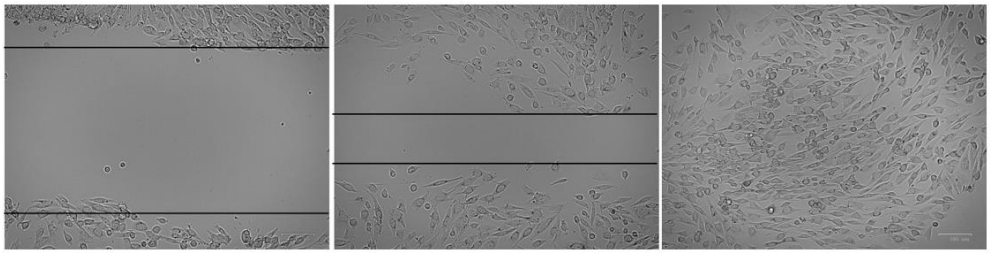


Figure 1: Demonstration of wound healing assay of Stigmasterol

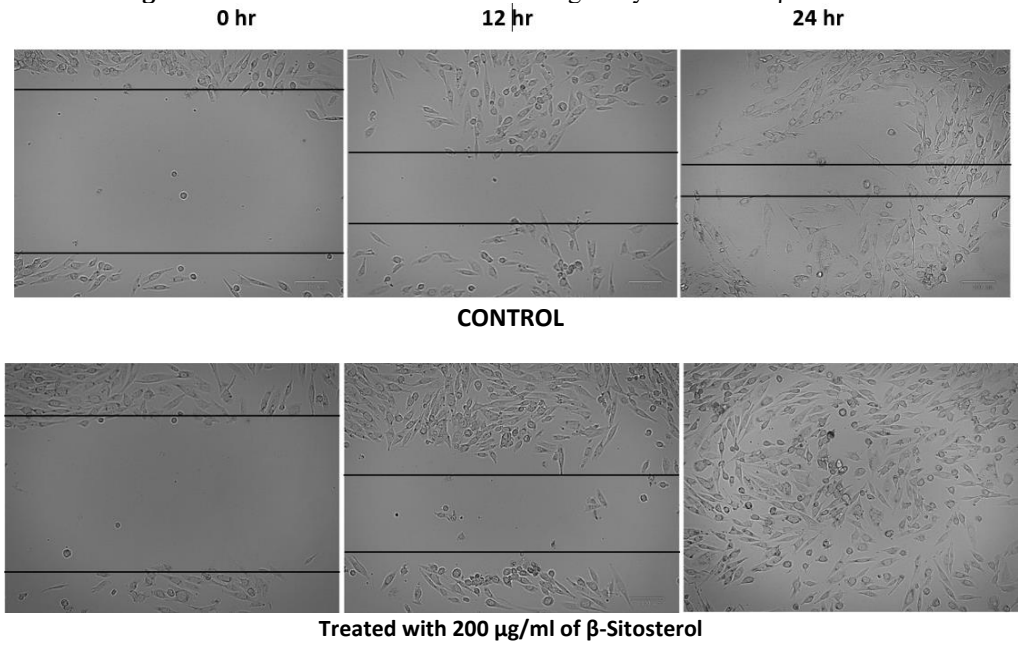


CONTROL



Treated with 200 µg/ml of Stigmasterol

Figure 2: Demonstration of wound healing assay with use of β-Sitosterol



4) DISCUSSION:

To investigate the effectiveness of phytosterol in the management of osteoarthritis various in-silico and in-vitro methods were being used. MG-63 cell line was used to determine their response towards the phytosterol treatment. Estimation of pharmacokinetic parameters of a compound helps to determine the absorption, distribution, metabolism, elimination and toxicity profile of a drug compound.

The prevalence of arthritis is solely characterized by the inflammation and joint destruction but the actual pathophysiology behind it is yet not known clearly. Osteoarthritis a most common type of arthritis affecting about 10% of men and 18% of women over 60 years of age. OA can manifest as monoarticular or polyarticular which is usually associated with pain, decreased mobility, stiffness and deformity(1,3). The diagnosis of OA is based on symptomatic presentation along with chronic joint pain and radiographic imaging shows joint space narrowing indicating bone cysts, subchondral sclerosis, osteophyte formation, and cartilage loss or bone degeneration.

Phytosterols are plant cholesterol having numerous therapeutic potential. Campesterol, stigmasterol, β-sitosterol are most common phytosterols which forms majority of human nutrition. Numerous of clinical studies conducted have shown that phytosterols have huge potentiating benefits but the was lack of evidence regarding the effect of phytosterol for the management of osteoarthritis. Therefore this study was designed and conducted to study the anti-osteoarthritic effect of phytosterol compounds(19).

pkCSM an online server for the estimation of physiochemical and pharmacokinetic parameter was being used. The absorption, distribution, metabolism, excretion and toxicology profile of the compounds were obtained using pkCSM. It provides a platform for the analysis and optimization of pharmacokinetic and toxicity properties implemented in a user-friendly, freely available web interface. It is a valuable tool to help medicinal chemists find the balance between potency, safety, and pharmacokinetic properties(8).Stigmasterol and β-Sitosterol both showed slight solubility in aqueous solvents with a very low Caco2 permeability. Intestinal absorption was low and the Blood Brain Barrier penetration was also found to nominal. CNS permeability was found to be nominal too. It also determines that phytosterols do not show toxicity and has a promising biological activity.

Use of Swiss ADME online server proved to be an essential tool determine physiochemical and pharmacokinetic property of the drug. It is a computer aided drug development web tool which helps to estimate absorption, distribution, metabolism, elimination of drugs along with lipophilicity and water solubility characteristics, bioavailability score, and medicinal chemistry properties(14,20). Determination of toxicity of a drug is very essential to avoid any occurance of adrverse effect. the use of OSIRIS property explorer helps to determine the toxicity and drug likeness. Drug likeness assess the capability of a drug molecule that can be formulated for oral consumption. The calculation of drug score determines the capability of chemical component to meet the demands of possible drug. It is the summation of molecular weight of a compound, its toxicity and c log P value. MOLINSPIRATION software determines the biological activity of the compound. Both stigmasterol and β-sitosterol showed bone disease treatment activity along with various other biological activity. PASS (Prediction of Activity Spectra for Substances) an oline web tool was used for determination of biological of phytosterol compounds. This tool helps the researchers to determine biological activity spectrum of the physiologically active components. This tool uses the substance structural formula to predict more than 300 pharmacological effect and biochemical pathways(12).

Alkaline phosphatase is one of the important biomarker for determination of the osteoblastic activity and helps us to understand bone cell biology. ALP plays a major role in bone mineralization. It is a ubiquitous enzyme which catalyzes the hydrolysis of phosphate ester in alkaline buffer and produces an organic radical and inorganic phosphate. Changes in ALP level and activity are associated with various disease states in the liver and bone. Various studies have put forward that ALP is present in plasma membrane of osteoblasts which buds out to form the matrix vesicles seen in developing bone. In our study we used MG-63 osteoblastic cell line in order to determine the effect of phytosterols on ALP activity. At the end of the assay we determined that the presence of phytosterol produced more OD at 405 nm with progression of time duration. With the results we inferred that the presence of phytosterol increases the ALP activity due to increase in the cell count which leads to increase breakdown of p-nitrophenylphosphate to yellow colored p-nitrophenol thereby increasing the OD of the solution. With this assay we inferred that phytosterols provide nourishment for the growth of osteoblastic cells and helps in bone mineralization.(21)

Migration is the key property of live cells which helps them for normal development, immune response and may also lead to disease progression such as cancer metastasis and inflammation. Migration of cells plays important role in wound healing(22,23). Wound healing is the process of reepithelization and is associated with angiogenesis (formation of new blood vessels) to supply enough nutrients essential for proliferation, growth and migration of the cells in order to replace the damaged cellular structure and tissue layer (24,25). In OA due to inflammation of the synovial joint, there is restriction in the movement of the bone and when movement of bone is produced it causes bruising of the nearby tissue and causes pain. In our study, wound healing assay with help of MG-63 cells was done in order to determine the effect of phytosterol in healing of wounds. The assay conducted with stigmasterol and β-sitosterol as test sample demonstrated that after 24 hours of exposure, the cells migrates toward the gap produced inform of wound. Therefore , the positive result obtained determined that phytosterols have epithelization stimulating effect(26)

5) CONCLUSION:

This study therefore confers that MG-63 osteoblastic cell line have shown positive response towards phytosterol treatment demonstrated by the *in-vitro* study. The *in-silico* study performed provides positive evidence indicating that phytosterols can be considered for the development of formulations which can be utilized for management of osteoarthritis. The dietary intake of phytosterols can be considered as non pharmacological approach for keeping the development and progression of osteoarthritis at its bay. This study provides evidence for further conduction of clinical and experimental studies with respect to use phytosterols for management of osteoarthritis and for the determination of its effectiveness.

6) ABBREVIATIONS:

OA (osteoarthritis), VEGFR-2 (vascular endothelial growth factor receptor-2), TPSA (total polar surface area), SAR (structural activity relationship), ALP (alkaline phosphatase) p-NPP (p- nitro phenyl phosphate), OD (optical density), DMEM (Dulbecco's modified eagle medium), OCT (organic cation transporter)

7) FUNDING:

No funding was acquired. The study was self funded by the research team.

8) AVAILABILITY OF DATA AND MATERIAL:

The paper is inclusive of all the data and information collected during the study.

9) AUTHORS CONTRIBUTION:

Gowri k and Chitra V contributed significantly in conception, design and execution ot the study. All laboratory experimentation and investigations was carried out by Gowri K under the supervision of Chitra V. Data collection, analysis, interpretation and article writing was done by Gowri K. Chitra V was incharge of article's conception, overall monitoring and final approval of the manuscript.

10) CONFLICT OF INTEREST:

The authors declare that there won't be any conflict of interest regarding the publication of the paper.

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