RESEARCH
O&G Forum 2024; 34 – 2s: 433 - 437

OSTEOBLASTIC DIFFERENTIATION MARKER GENE EXPRESSION STATUS IN RESPONSE TO GINGEROL TREATMENT

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Abstract

Introduction:
Using a bioactive compound named Gingerol which is derived from a traditional medicinal plant has a wide variety of therapeutic and medicinal use mainly in producing bone formation and osteoblastic marker gene expression.Mesenchymal stem cells(MSCs) helps in promoting the formation and mineralisation of bone.

Aim:
The aim of this study is to determine the Osteoblastic differentiation marker gene expression status in response to gingerol treatment and identify the osteogenic potential at non-toxic concentration of gingerol.

Materials and Method:
Materials used are the osteogenic cell line,gingerol and process it through MTT Assay,RT-PCR technique.

Result:
Three different analysis were taken I.e:Cytotoxicity assessment of Gingerol on osteoblast by MTT Assay 24 h,Col-1 gene expression in 7 days of osteogenic medium and Runx2 gene expression in 7 days of osteogenic medium,Results have shown that gingerol was found to be non-toxic concentration at 2.5 mM.

Conclusion:
From this preliminary study we can conclude that gingerol is non-toxic till 2.5mM and cells were able to survive in those concentration.So it promotes the osteoblastic regulation at specific concentration and upregulate the expression of COL 1 and Runx2 enzymes.It is speculated from this study that gingerol is used for used for bone related therapies.

Keywords:Gingerol,Osteoblast,MTT Assay,RT-PCR,MSC,COL 1,Runx2.

Introduction:
Osteoblastic differentiation refers to the process by which mesenchymal stem cells (MSCs) develop into osteoblasts, which are the cells responsible for bone formation and mineralization.Osteoblasts is integrated by the process of ossification.Mesenchymal stem cells are the building factor of osteoblast.Process involved in ossification are proliferation,mineralisation and matrix maturation(1).Key enzymes which are regulating the osteoblast are *runx2* enzymeOsteoblast are enlarged through circle osteocyte.In principle, a molecule responsible for promoting the formation of bone cells should meet three requirements: it should be produced in early bone cell precursors, regulate the activity of numerous genes specific to bone cells, and play a crucial role in the process

of bone cell development both in laboratory settings and in living organisms. So far, only a single transcription factor named Cbfa1 or Runx2 has been found to meet all of these criteria(2).
Multipotent cells found in the bone marrow have the ability to transform into various cell types such as fibroblasts, adipocytes, and osteoblasts (3).A recent discovery revealed that when bone marrow cells are exposed to a type I collagen matrix, they undergo a process of differentiation that leads specifically to the development of osteoblasts(4).The levels of alkaline phosphatase and osteopontin gene expression progressively rose over time during the process of osteoblastic differentiation. Cells that formed mineralized tissues showed the expression of osteocalcin and bone sialoprotein genes, but this expression occurred only once the cells had reached the stage of tissue mineralization(5).
Gingerol is a bioactive compound found in ginger and a widely used spice and traditional medicinal plant(6). It has been studied for its potential therapeutic properties, including its effects on bone health.Gingerol treatment has demonstrated the potential to promote osteoblastic differentiation and enhance bone formation through the modulation of osteoblastic marker gene expression(7).
Throughout history, humans have utilized ginger, scientifically known as *Zingiber officinale,* for its medicinal qualities and as a flavorful spice to enhance culinary experiences(8).Ginger is rich in various phenolic compounds, including gingerol, shogaol, and paradol, which possess beneficial properties such as antioxidants, anti-tumor effects, and anti-inflammatory capabilities(9).In recent years, numerous researchers have concentrated their efforts on investigating the potential of ginger and its components in improving various ailments. These studies have yielded compelling scientific evidence supporting the health advantages associated with ginger.

The aim of this study is to determine the Osteoblastic differentiation marker gene expression status in response to gingerol treatment.
Objectives are to identify the non-toxic concentration of gingerol and to elucidate the osteogenic potential of gingerol at a non toxic concentration in osteoblast.

Materials and Methods:
Study was conducted in vivo in Saveetha Dental College,Chennai.The duration of the study was
General:
Osteoblastic cell lines(*col-1*,*Runx-2)*was derived from NCCS,Pune.Both genes cell lines provide valuable tools for investigating osteoblast biology,bone mineralization,and the regulation of bone related genes.*col-1* (Collagen type-1) is a predominant cell line which was used as model for studying bone formation and extracellular matrix production.Derived from primary cell culture of osteoblast from human or animal bone tissue which provide immortalization techniques or by transforming primary osteoblasts.
*RUNX-2(*Runt-related transcription factor 2) cell line used to study the molecular mechanism involved in osteoblast differentiation and bone formation.This gene was derived from primary cultures of osteoblast and immortalized or transformed to establish a stable cell line expressing *RUNX-2* protein.

Compound
Gingerol was a compound derived from ginger(*Zingiber officinale)*,a flowering plant that is widely used as a spice and traditional medicine.Chemical structure composed of phenolic ring with a hydroxyl group(-OH) and an unsaturated aliphatic chain.The most abundant is 6-gingerol but 8-gingerol and 10-gingerol can also be used in smaller groups.

MTT ASSAY
Cell Culture: Human osteoblastic cells (cell line) was cultured in appropriate growth medium supplemented with Fetal Bovine Serum (FBS) and antibiotics. Cells will be maintained in a controlled incubator at 37°C with 5% CO2.
Experimental Groups: Cells was divided into three groups: Control (untreated), Gingerol 1mM, Gingerol 2.5mM, and Gingerol 5mM.
Treatment: Cells had been seeded in 96-well plates and allowed to adhere overnight. The following day, cells was being treated with the respective concentrations of gingerol for 24 hours.
MTT Assay: After the treatment period, the medium has been aspirated, and cells washed with PBS. MTT reagent was added to each well and the plate had been incubated for an additional 4 hours to allow formazan crystals to form.
Formazan Extraction: Formazan crystals solubilized using a suitable solvent (e.g., DMSO). The absorbance of the resulting solution has been measured at an appropriate wavelength using a microplate reader.

RT-PCR:
Cell Culture: MG-63 cells was cultured in appropriate growth medium supplemented with osteogenic inducers, such as ascorbic acid and β-glycerophosphate, in a controlled incubator at 37°C with 5% CO2.
Experimental Groups: Cells has been divided into three groups: Control (untreated), Gingerol 1mM, and Gingerol 2.5mM.
Treatment: MG-63 cells was seeded in tissue culture plates and cultured in osteogenic medium. After reaching appropriate confluence, cells was treated with 1mM and 2.5mM concentrations of gingerol for 7 days.
RNA Extraction: Following the treatment period, total RNA extracted from cells using a commercially available RNA extraction kit according to the manufacturer's instructions.
cDNA Synthesis: Reverse transcription of RNA to cDNA was performed using a reverse transcription kit with random primers.
Real Time RT-PCR: Quantitative analysis of gene expression had been conducted using Realtime RT-PCR. Specific primers for Runx2, Col-I, and reference gene (e.g., GAPDH) was designed and optimized.
PCR Amplification: Real Time RT-PCR reactions was carried out using cDNA, gene-specific primers, and a suitable master mix. Amplification conditions was optimized for efficient and specific amplification.
Statistical Analysis: Data has been analyzed using appropriate statistical methods (e.g., t-test or ANOVA) to determine significant differences in gene expression between experimental groups.

Results:
Figure.1:

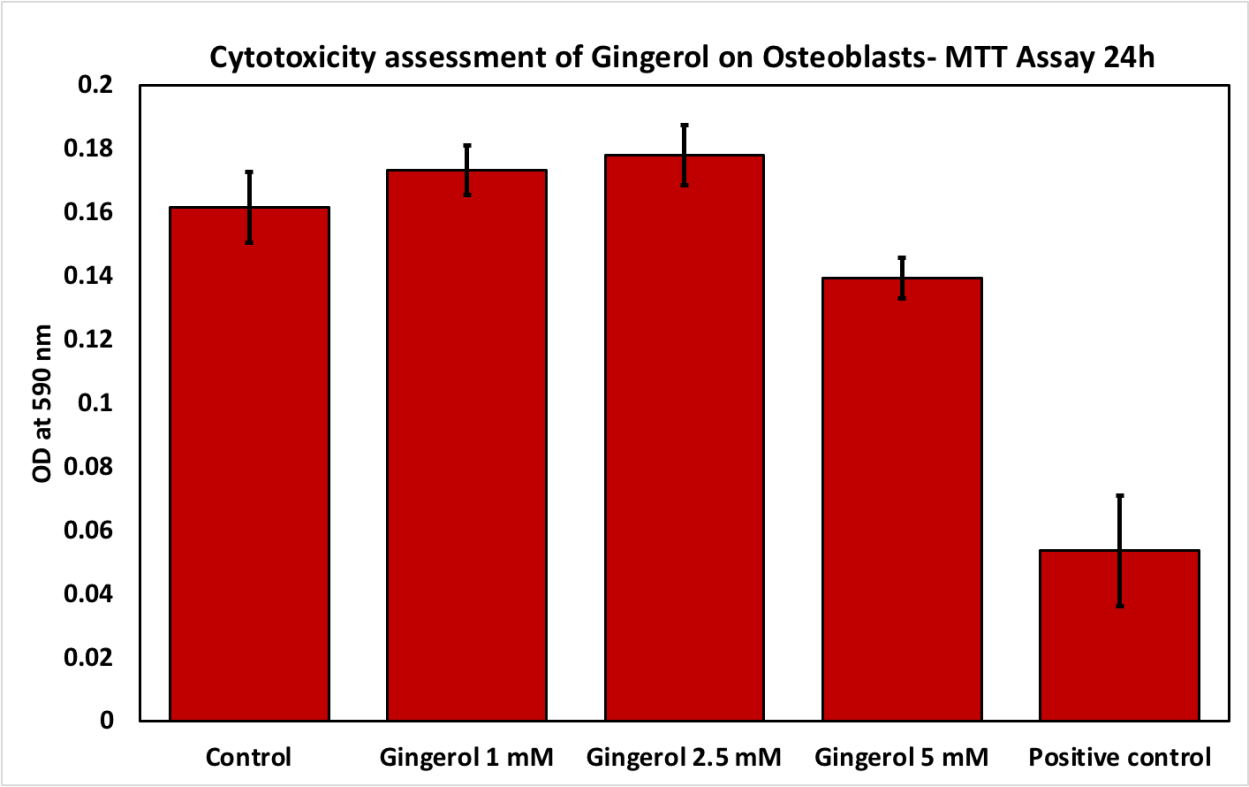


Figure.2:

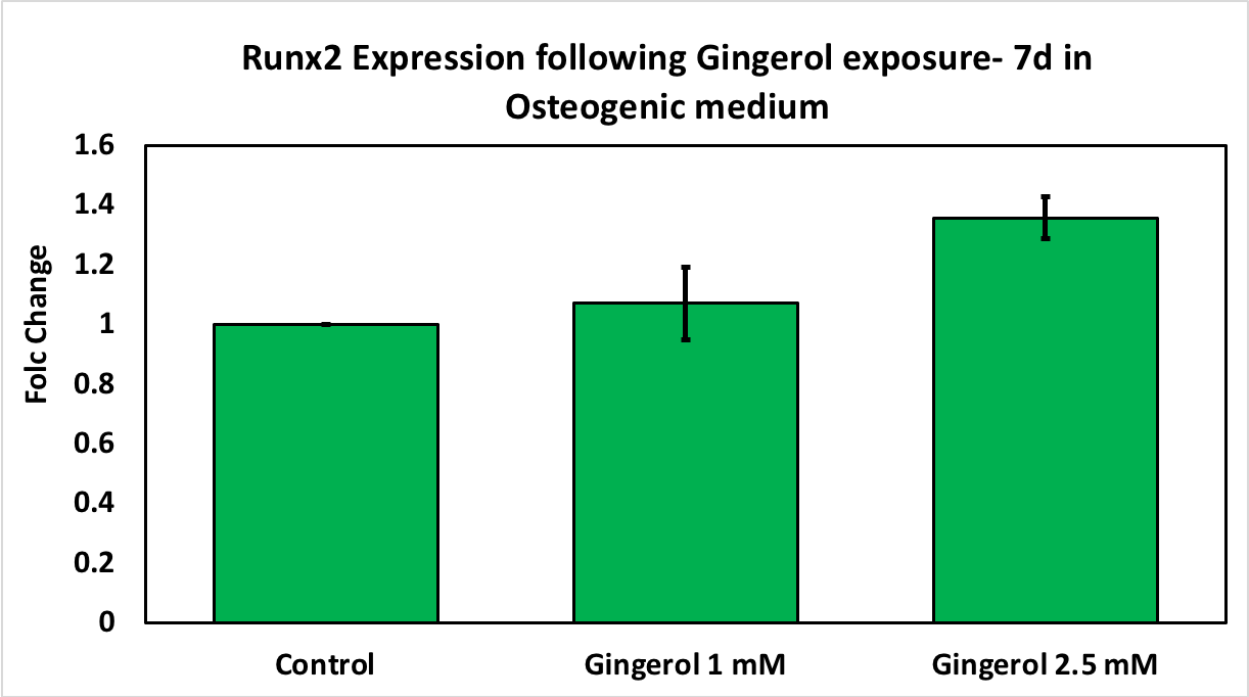
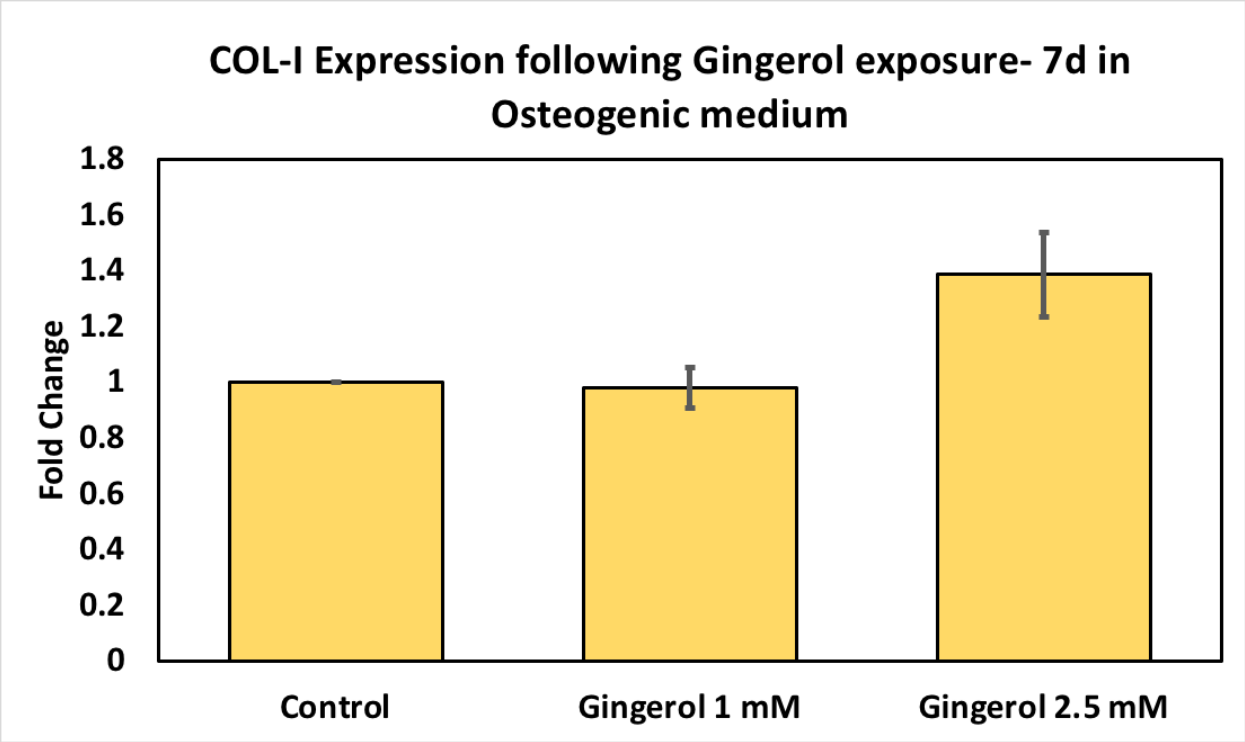


Figure.3:



In this conducted research, the potential of gingerol to induce osteogenesis was explored using human osteoblastic (MG-63) cells. These cells were subjected to varying concentrations of gingerol, namely 1mM and 2.5mM, for a duration of 7 days in a controlled in vitro osteogenic environment. The analysis employed real-time PCR to evaluate the expression levels of pivotal osteogenic genes, specifically Runx2 and Col-I.

The outcomes derived from the real-time PCR analysis notably demonstrated a substantial increase in the expression of both the Runx2 and Col-I genes within cells exposed to 2.5mM gingerol. When compared against the control group (cells not exposed to gingerol) as well as the 1mM gingerol-treated group, this heightened gene expression implies that gingerol holds the potential to augment osteogenic differentiation and the deposition of extracellular matrix components in MG-63 cells.

Discussion:

The outcomes of this research contribute to the existing knowledge about gingerol's osteogenic properties. Runx2 is a crucial transcription factor known for its role in osteoblast differentiation and bone formation. The heightened expression of Runx2 in response to 2.5mM gingerol implies that gingerol could accelerate bone formation by promoting osteoblast differentiation.

Likewise, the increased expression of Col-I, a significant component of the bone matrix, supports the idea that gingerol might enhance the synthesis of the extracellular matrix, crucial for bone regeneration. This is consistent with earlier studies that have shown the positive impact of gingerol on osteogenic markers and genes related to bone health(10).The part of ginger and its constituents in enhancing diseases has been the center of think about within the past two decades by numerous analysts who give solid logical prove of its wellbeing advantage. This audit examines inquire about discoveries and works given to gingerols, the major impactful constituent of ginger, in tweaking and focusing on signaling pathways with ensuing changes that enhance, invert or anticipate inveterate infections in human ponders and creature models(9).The carbon chain length has also played a significant role in making 10-gingerol as the most potent among all the gingerols.This study justifies the use of dry ginger in traditional systems of medicine(11).

Gingerol at 2.5mM is being suitable for the osteoblastic differentiation and enzyme like runt-related transcription factor 2(RUNX2) and Collage type-1(COL-1) were upgraded.The Bone Morphogenic protein(BMP) stimulated the RUNX2 had bone specific protein and upgraded protein kinase activity(12).The findings indicate that gingerol has the potential to influence the osteoblastic differentiation process and promote bone formation. The upregulation of key marker genes, such as RUNX2, OCN, and ALP, suggests that gingerol treatment can enhance osteoblast commitment, maturation, and mineralization.The observed effects of gingerol on osteoblastic differentiation marker gene expression may be attributed to its ability to modulate various signaling pathways and growth factors.RUNX2, gingerol treatment also increases the expression of OCN, a marker of mature osteoblasts involved in bone mineralization.ALP is involved in the mineralization process and its upregulation by gingerol implies enhanced osteoblastic differentiation and maturation.These enzymes also stated in the regulation of cell cycle where it significantly increase after mitosis(13).Investigation are needed to explore the underlying mechanisms through which gingerol influences osteoblastic differentiation and to determine the optimal dosage and treatment duration for its potential therapeutic use.In vivo studies and clinical trials are necessary to validate the in vitro findings and assess the overall effectiveness and safety of gingerol in promoting bone health.The extracellular matrix,Collagen provide structural support to other cells and is more fibrous protein and provide a ongoing fibroplasia in other area(14).

Scope of future research:

Investigation are needed to explore the underlying mechanisms through which gingerol influences osteoblastic differentiation and to determine the optimal dosage and treatment duration for its potential therapeutic use.In vivo studies and clinical trials are

necessary to validate the in vitro findings and assess the overall effectiveness and safety of gingerol in promoting bone health.

Conclusion:
From this preliminary study we can conclude that gingerol is non-toxic till 2.5mM and cells were able to survive in those concentration.So it promotes the osteoblastic regulation at specific concentration and upregulate the expression of COL 1 and Runx2 gene enzymes.It is speculated from this study that gingerol is used for used for bone related therapies.

Author Contribution:
All author have equally contributed to the research.

Ethical clearance number:
Since it is an in vitro study,ethical clearance is not needed.

Conflict of Interest : Nil

Source of Funding:
1.Saveetha Institute of Medical and Technical Science
2.Saveetha Dental College and Hospitals
3.Saveetha University
4.KPC Pipes

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