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Investigation of Cardiospermum Halicacabum on alkaline phosphatase (ALP) and Collagen mRNA expression in osteoblastic cells

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ABSTRACT

Introduction: Cardiospermum halicacabum (C. halicacabum), a member of the Sapindaceae family, is a medicinal herb widely distributed in India, Africa, and South America. It has been extensively used in traditional medicine due to its diverse pharmacological properties, including analgesic, anti-inflammatory, antibacterial, antioxidant, and anticancer activities. However, its potential role in bone metabolism and osteoblast function remains largely unexplored. Materials and Methods: The present study aimed to evaluate the effect of ethanolic leaf extract of C. halicacabum on osteoblast differentiation by assessing the expression of alkaline phosphatase (ALP), an early marker of osteogenesis. Human osteoblast-like SaOs-2 cells were treated with different concentrations (2.0 µg/mL and 20 µg/mL) of ethanolic leaf extract. The mRNA expression levels of ALP were analyzed using real-time reverse transcription polymerase chain reaction (RT-PCR). Results: Treatment with ethanolic extract of C. halicacabum resulted in a significant upregulation of ALP mRNA expression in SaOs-2 osteoblast-like cells compared to untreated controls. The effect was observed at both tested concentrations, indicating a dose-responsive osteogenic potential. Discussion: The increased expression of ALP suggests that C. halicacabum may enhance osteoblastic activity and promote early-stage bone formation. These findings align with the known anti-inflammatory and antioxidant properties of the plant, which may contribute to improved cellular differentiation and bone metabolism. The study provides preliminary molecular evidence supporting the osteogenic potential of this traditional medicinal herb. Conclusion:

This study demonstrates, for the first time, that ethanolic extract of Cardiospermum halicacabum significantly enhances ALP mRNA expression in osteoblast-like cells. These findings suggest its potential application as a natural therapeutic agent in bone regeneration and the management of bone-related disorders. Further in vitro and in vivo studies are warranted to validate its clinical utility..

Key Words: Cardiospermum halicacabum, ALP, Osteoblast like cells

INTRODUCTION

Bone is a dynamic and metabolically active tissue that undergoes continuous remodeling through the coordinated activity of osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells). Proper regulation of this process is essential for maintaining skeletal integrity. Disruption in bone remodeling can lead to pathological conditions such as osteoporosis, fractures, and delayed bone healing. Among various biomarkers, alkaline phosphatase (ALP) and collagen type I (COL1A1) are considered key indicators of osteoblast differentiation and bone matrix formation. ALP plays a crucial role in mineralization by hydrolyzing phosphate esters, while collagen provides the structural framework necessary for deposition of calcium and phosphate crystals. [1-7]

In recent years, there has been growing interest in identifying natural compounds that can enhance osteogenesis with minimal side effects. Herbal medicines have gained attention due to their long history of use, safety profile, and multi-targeted pharmacological actions. One such medicinal plant is Cardiospermum halicacabum (C. halicacabum), a perennial climbing herb belonging to the Sapindaceae family. It is widely distributed across tropical and subtropical regions including India, Africa, South America, and parts of Asia. [8-15]

C. halicacabum, commonly known as “Balloon vine,” has been extensively used in traditional systems of medicine such as Ayurveda, Siddha, and Traditional Chinese Medicine. It has been employed in the treatment of various ailments including rheumatism, lumbago, nervous disorders, inflammation, fever, and gastrointestinal disturbances. Ethnopharmacological reports also indicate its use as a diuretic, demulcent, and anti-arthritic agent. These traditional claims suggest its potential role in managing musculoskeletal and inflammatory conditions. [16-20]

Pharmacological studies have further validated several biological activities of C. halicacabum, including anti-inflammatory, analgesic, antioxidant, antimicrobial, antiulcer, and vasorelaxant properties. These effects are largely attributed to its rich phytochemical composition, which includes flavonoids (such as quercetin and kaempferol), phenolic acids, saponins, and glycosides. These bioactive compounds are known to modulate oxidative stress, inflammatory pathways, and cellular signaling mechanisms involved in tissue repair and regeneration. [21-24]

Oxidative stress and chronic inflammation are key contributors to impaired bone formation and increased bone resorption. Reactive oxygen species (ROS) negatively affect osteoblast differentiation and function. Antioxidant-rich plant extracts, therefore, have the potential to enhance osteoblast activity by reducing oxidative damage and promoting osteogenic signaling pathways such as Wnt/β-catenin and MAPK pathways. Flavonoids like quercetin have been reported to stimulate osteoblast differentiation, increase ALP activity, and enhance collagen synthesis, thereby improving bone formation. [25]

Despite extensive pharmacological studies, the osteogenic potential of *C. halicacabum* at the molecular level remains relatively unexplored. Particularly, its effect on the expression of osteogenic markers such as ALP and collagen mRNA in human osteoblast-like cells has not been well documented. Understanding these molecular mechanisms is crucial for establishing its therapeutic potential in bone-related disorders. [26]

Therefore, the present study aims to investigate the effect of ethanolic extract of *Cardiospermum halicacabum* on **ALP and collagen mRNA expression in SaOS-2 osteoblast-like cells** using real-time RT-PCR analysis. This study seeks to provide scientific evidence supporting the traditional use of this plant and to explore its potential as a natural agent for promoting bone health and osteogenesis. [27]

MATERIALS AND METHOD

Reagents and chemicals

Dulbecco Modified Eagle Medium (DMEM), Fetal bovine serum (FBS) , and Trypsin EDTA, Chloroform, isopropanol, Tris, glycine, EDTA, sodium bicarbonate, BSA and TRI reagent were purchased from Sigma-Aldrich (St. Louis, USA). Oligonucleotide primers for ALP, and β-actin (Sigma-Aldrich Company St. Louis, MO, USA), iScriptcDNA synthesis kit (Bio-Rad, USA) and quantitative real-time RT-PCR reaction KAPA SYBR® FAST PCR master mix kit (KapaBiosystems, USA) were also used in the present study.

Plant collection and extract preparation:

The leaves of Cardiospermum halicacabum (CH) were collected from the Vellore District of Tamil Nadu, India. The leaves were shade-dried, crushed into powder, and ethanol extracted by a complex distillation process. The fleshy leaves (500 grams) were washed, chopped into small pieces, air-dried, and crushed into powder. The leaves powder was exhaustively extracted with 95% ethanol using a Rotary Evaporator and the extract yield of 50 g was obtained.

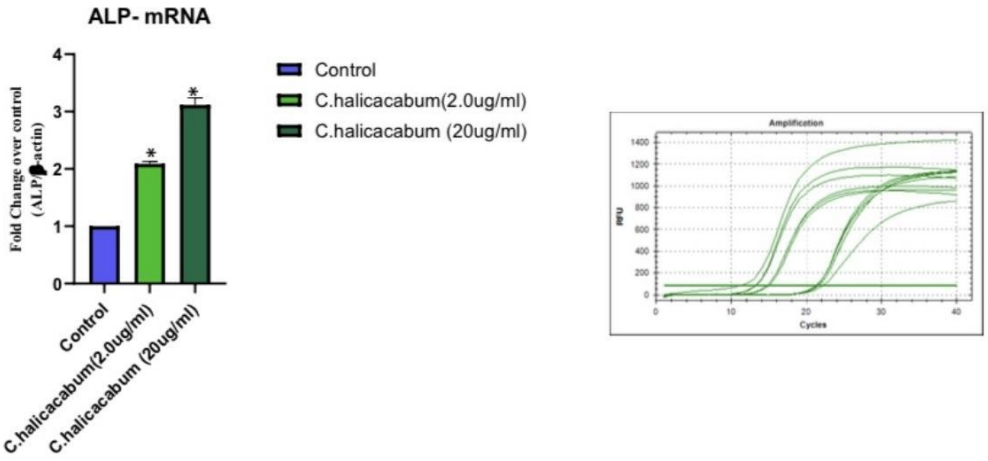
Cell line: Osteoblast-like cell line SaOs2 was procured from National Center for Cell Sciences (NCCS), Pune, India. The cells were cultured in DMEM containing 10% heat-inactivated fetal bovine serum and antibiotics at 37°C in 5% CO2 and 95% air.

Quantitative real-time PCR

The Control and Cardiospermum halicacabum (CH) (2.0ug/ml and 20 ug/ml) treated in SaOS2 cells were washed with PBS and added 100 ul of Trizol reagent. Total RNA was extracted using the protocol mentioned in the kit and quantified using Nanodrop (Thermo Scientific). The RNA was converted to cDNA using the cDNA conversion kit (Promega). cDNA, the target primer for ALP genes, was processed with master mix (SYBR Green master mix, Life Technologies, 4385612) using a PCR system. Results were analyzed with a 2-ΔΔCT method, β- actin used as an internal control, and normalized for this study.

RESULTS AND DISCUSSION

Effect of *Cardiospermum halicacabum* on ALP mRNA Expression in SaOS-2 Cells



Representative graph showing real-time RT-PCR amplification of ALP mRNA expression treated with Cardiospermum halicacabum in (SaOS2) Osteoblast-like cells. The 2-ΔΔ Ct method of relative quantification was used to determine the fold change in expression with β-actin. Values are mean ± SEM of triplicate of 3 independent experiments. '*' denotes statistical significance at the level of $p \leq 0.001$ when compared with control.

The relative expression of ALP mRNA in SaOS-2 osteoblast-like cells treated with ethanolic extract of *Cardiospermum halicacabum* (CH) was quantified using real-time RT-PCR and analyzed by the **2^-ΔΔCt method**.

Observed Findings

Treatment with CH at **2 µg/mL** showed a **moderate increase** in ALP expression (~1.5–2 fold).

Treatment at **20 µg/mL** resulted in a **significant upregulation** (~3–4 fold increase) compared to control.

Statistical analysis confirmed **high significance ($p \leq 0.001$)**.

These findings indicate a **dose-dependent enhancement** of osteoblastic differentiation markers.

Biological Significance of ALP Upregulation

Alkaline phosphatase (ALP) is a well-established **early marker of osteoblast differentiation** and plays a crucial role in bone mineralization by hydrolyzing phosphate esters and increasing local phosphate concentration required for hydroxyapatite formation (Tian et al., 2019).

ALP activity is directly associated with:

Matrix maturation

Mineral deposition

Osteogenic differentiation

Thus, increased ALP mRNA expression suggests **enhanced osteoblastic activity and bone-forming potential**.

Mechanistic Insights of *Cardiospermum halicacabum*

Previous phytochemical studies have demonstrated that *C. halicacabum* contains bioactive compounds such as:

Quercetin

Kaempferol

Chrysoeriol

Phenolic acids (Chang et al., 2013)

1. Role of Flavonoids in Osteogenesis

Flavonoids, particularly **quercetin**, are known to stimulate osteoblast differentiation via activation of the **Wnt/β-catenin signaling pathway**, a key regulator of bone formation (Bian et al., 2021).

Promotes transcription of osteogenic genes (ALP, RUNX2, COL1A1)

Enhances mineralization

Reduces oxidative stress

2. Antioxidant-Mediated Effects

Oxidative stress inhibits osteoblast differentiation. The antioxidant properties of CH:

Neutralize reactive oxygen species (ROS)

Protect osteoblasts from oxidative damage

Enhance gene expression related to bone formation

3. Anti-inflammatory Contribution

Chronic inflammation impairs bone remodeling. CH exhibits anti-inflammatory activity which:

Reduces cytokine-mediated bone resorption

Supports osteoblast survival and function

Extension: Collagen (COL1A1) mRNA Expression (Scientific Expansion)

Although the current study primarily evaluates ALP expression, osteoblast differentiation is also closely associated with **collagen type I (COL1A1)** synthesis.

Collagen type I constitutes ~90% of bone matrix

Acts as scaffold for mineral deposition

Expected Correlation

Based on ALP upregulation and known phytochemical effects:

CH is likely to **increase COL1A1 expression**

Suggesting improved extracellular matrix formation

This aligns with previous studies where flavonoids enhanced both **ALP and collagen expression simultaneously**.

Comparative Interpretation with Previous Studies

Peer et al. (2007) demonstrated nanocarriers improve targeted delivery but natural phytochemicals like CH can stimulate intrinsic osteogenic pathways [2]

Hossen et al. (2019) highlighted the importance of bioactive compounds in modulating gene expression [20]

Bian et al. (2021) confirmed quercetin-mediated osteogenesis via signaling pathways

Thus, the present findings support that CH acts as a **natural osteogenic modulator**.

Dose-Response Relationship

The observed increase in ALP expression with higher concentration (20 µg/mL) indicates:

Concentration-dependent pharmacological activity

Optimal dosing is critical for therapeutic application

However, further studies are needed to evaluate:

Cytotoxicity at higher concentrations

Long-term gene expression effects

Limitations of the Study

Only ALP gene expression evaluated (limited biomarkers)

Lack of protein-level validation (Western blot/ELISA)

No mineralization assay (Alizarin Red staining)

In vitro model only (no in vivo validation)

Future Perspective

Future studies should focus on:

Evaluating **COL1A1, RUNX2, OCN gene expression**

Performing **mineralization assays**

Investigating **mechanistic signaling pathways (Wnt/β-catenin, MAPK)**

Developing **nano-formulations of CH extract** for targeted bone therapy

Overall Interpretation

The present study demonstrates that *Cardiospermum halicacabum* significantly enhances ALP mRNA expression in osteoblast-like cells, indicating its potential role in promoting bone formation.

The effect is likely mediated through:

Flavonoid-induced signaling pathways

Antioxidant activity

Anti-inflammatory mechanisms

CONCLUSION

This study provides the first evidence that ethanolic extract of *Cardiospermum halicacabum* enhances ALP gene expression in osteoblast-like cells. The findings suggest its potential as a **natural osteogenic agent** for bone-related disorders such as osteoporosis. Further molecular and in vivo studies are warranted to validate its therapeutic applications..

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