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EFFECT OF CARDIOSPERMUM HALICACABUM ON RUNX2 mRNA EXPRESSION IN OSTEOBLAST CELLS

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ABSTRACT:
Cardiospermum halicacabum (C. halicacabum) is a Sapindaceae herb native to India, Africa, and South America that has long been utilised in traditional medicine. According to folk medicine, these plants have a variety of qualities, including analgesic, anti-inflammatory, antibacterial, antioxidant, and antitumor actions. Runx2 has a crucial role in osteoblast differentiation. It is involved in the regulation of the expression of osteocalcin, the only gene expressed in osteoblasts and in no other extracellular matrix (ECM)-producing cell.The aim of the present study was to determine the effects of ethanolic extract of *Cardiospermum halicacabum* on the expression of Runx2 in the (SaOs2) human osteoblast-like cells.The Osteoblast-like cells SaOs2 was treated with different doses of ethanolic leaf extract of C.halicacabum (0.001, 0.01, 0.1, 1, 10, 100 and 1000 µg/ml) and assessed for cell proliferation. Cell proliferation was studied by MTT assay at 24 and 48 h. Then the cells were treated with either vehicle (0.01% DMSO) or C.halicacabum (2.0 and 20 µg/ml) and assessed for the mRNA and protein expression of major transcription factor Runx2 in osteoblast was studied by real-time RT-PCR and western blot analyses, respectively. Our findings demonstrate that *C.halicacabum* increased the proliferation and mRNA expression of Runx2 in osteoblast-like cells. In conclusion, the present study demonstrates for the first time that ethanolic extract of *C. halicacabum* facilitates the Runx2 mRNA expression in Osteoblast cells.

KEYWORDS: Cardiospermum halicabum, Runx2, Osteoblast like cells,medicine

1. INTRODUCTION: Numerous chemical compounds that regulate a variety of biological functions and have a variety of beneficial effects on human health can be found in natural products derived from medicinal plants or herbs (1)(2).According to a report from the World Health Organization, 80% of consumers worldwide are confident that plant medicines may be a viable option for their health and that thousands of plant species can offer intervention strategies (3,4). *Cardiospermum halicacabum* is a plant in the Sapindaceae family that is native to India, Africa, and South America . It has been used for a long time in Chinese medicine (5). Runx2 has a crucial role in osteoblast differentiation. It is involved in the regulation of the expression of osteocalcin, the only gene expressed in osteoblasts and in no other extracellular matrix (ECM)-producing cell type .

In animal models, a variety of CH pharmacological actions have been investigated.In RAW264.7 cells, ethanolic extract inhibits LPS-induced COX-2, TNF-, and iNOS expression due to its anti-inflammatory activity (6). A family of transcription factors that is identical to the Drosophila runt is encoded by the mammalian Runx (runt-related transcription factor x) genes, which include Runx1, Runx2, and Runx3. Regulation of Runx gene expression and protein function occurs at multiple levels through multiple signalling pathways (7). During bone development, the temporal and spatial expression patterns of Runx2 are regulated by cytokines, growth factors, and hormones, including TGF-β, BMP, FGF, sonic hedgehog, vitamin D3, and oestrogen (8) (9). The role of microRNAs (miRs) in the regulation of osteogenesis is a fascinating new development (10). These are 18–25 nucleotides long non-coding RNAs that inhibit gene expression by either promoting the degradation of the target mRNAs or inhibiting their translation. They do this by binding to the 3′-UTR of mRNAs for specific target genes (11).

2.MATERIALS AND METHODS:

2.1 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.

2.2 Chemicals: Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Trypsin-EDTA, foetal bovine serum (FBS), antibiotics-antimycotics, Dulbecco's modified Eagle's medium (DMEM), and phosphate-buffered saline (PBS) were purchased from Gibco, Canada. Trizol (Invitrogen), Chloroform, Isopropanol, Ethanol (SRL, Mumbai), cDNA Synthesis kit was purchased from Takara, South Korea and Real-time PCR kit (Syber Green) was purchased from Invitrogen, USA. All the chemicals used were extra pure of analytical grade.

2.3 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 foetal bovine serum and antibiotics at 37°C in 5% CO2 and 95% air.

2.4 Cell Viability: SaOs2 Osteoblast like- cells were treated with either vehicle (0.01% DMSO) or different doses of ethanolic leaf extract of *C.halicacabum* (0.0001, 0.001, 0.01, 0.1, 1, 10, 100 and 1000 µg/ml) and assessed for cell proliferation by MTT assay at 24h.

2.5 Quantitative real-time PCR: The Control and *Cardiospermum halicacabum* (CH) (2.0ug/ml and 20 ug/ml) treated for 24 h 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 Runx2 genes. Primers details of the Runx2 (Runx2-124 bp , Gene Accession Number: NM_001278478.2, Forward primer: 5'TGTTCCAAAGACTCCGGCAA 3' and Reverse primer: 5'AGTTTGATGAGGCCGACTGC 3') was processed with master mix (SYBR Green master mix, Life Technologies, 4385612) using a PCR system. Results were analysed with a 2-ΔΔCT method, β- actin used as an internal control, and normalised for this study. Primers details of the β-actin (β-actin-100 bp, Gene Accession Number: NM_001101.5 Forward primer 5'CAACACAGTGCTGTCTGGTGGTA 3' and Reverse primer 5'CTTGATCTTCATGGTGCTGGGA 3').

3. RESULTS: The cell Viability of SaOS2 (Osteoblast-like cells) was measured by MTT assay and the results are shown in Fig 1. The viability of cells could change after 24h treated with C. halicacabum at 100µg/ml concentrations.

In the present study *C. halicacabum* significantly increased (2.0-fold and 3.0-fold) the Runx2 mRNA expression in 2.0µg/ml and 20 µg/ml of *C. halicacabum* treated groups when compared to control (Fig 2).

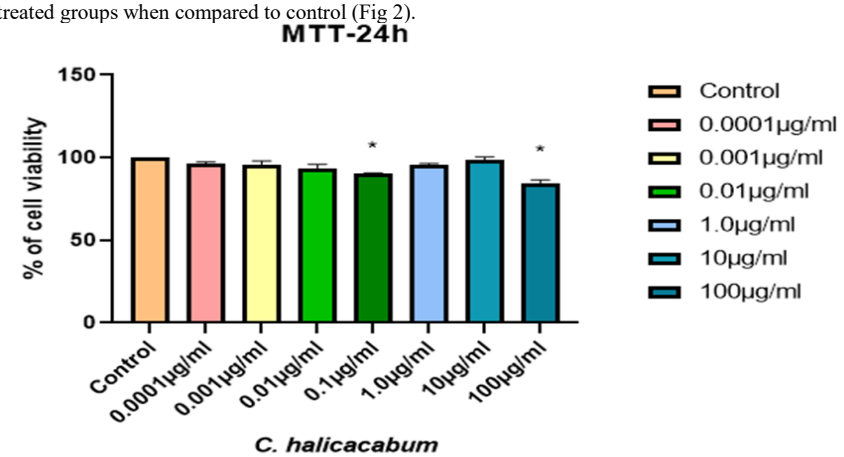

Figure 1: Effect of C.halicacabum on the SaOS2 Osteoblast-like cell viability at 24 h. SaOS2 cells were treated with vehicle (0.01% DMSO) or different doses of *C.halicacabum* and assessed for cell viability by MTT assay. Each bar represents the mean ± SEM of 6 observations. * $P < 0.05$.

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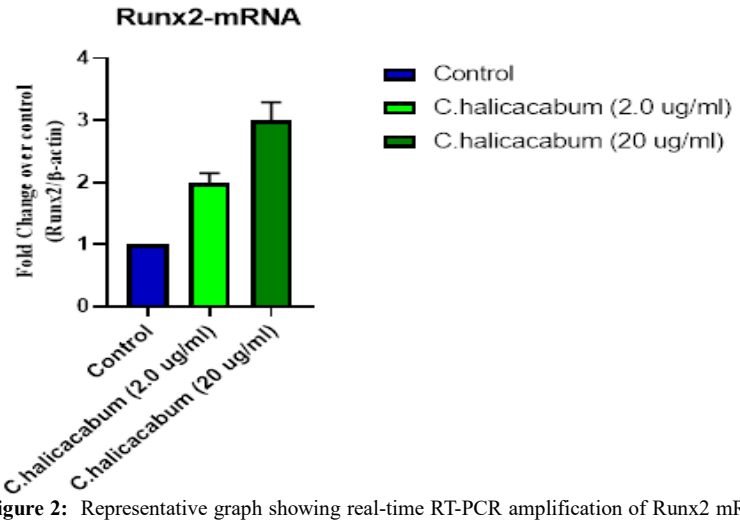

Figure 2: Representative graph showing real-time RT-PCR amplification of Runx2 mRNA expression treated with *C. halicacabum* in (SaOS2) Osteoblast-like cells. The 2-ΔΔ Ct method of relative quantification was used to determine the fold change in expression with β-actin. Values are mean ± SEM of triplicate of 3 independent experiments. '*' denotes statistical significance at the level of $p \leq 0.001$ when compared with control.

4. DISCUSSION:

Runx2 plays a vital and diversified role depending on the cellular origin. It acts as an osteogenic differentiation factor in bone cells. Upon various stimuli, Runx2 interacts with different proteins resulting in positive or negative regulation of its target genes. The post translational modification to Runx2, especially phosphorylation, dictates its functional interaction with other proteins controlling osteoblast differentiation (12). It is important to note that the list of proteins interacting with Runx2 keeps growing. This will pave the way for additional regulatory pathway analysis, which could facilitate the development of alternate and effective therapies for various bone and bone related diseases (13). Runx2 is a transcription factor that has been described as an important master regulatory gene controlling osteogenesis. Runx2 regulates several genes like osteocalcin, type I collagen, and ALP while promoting osteogenic differentiation and subsequent matrix mineralization. In the present study, the effects of *C.halicacabum* on the expression of Runx2 at RNA levels were studied. *C.halicacabum* contains Chrysoeriol, Quercetin, Kaempferol, and Hydroquinone. Incidentally, many of these constituents were already reported to have regulatory effects on some of the parameters in osteoblasts (14).

SaOS2 represents one of the cell lines available to specifically study gene regulation by Runx2 since expression of neither Runx1 nor Runx3 was detected in these cells (9). The rise in Runx2 mRNA expression clearly shows that *C. halicacabum* increases the functional activity of osteoblasts. Runx2 mRNA expression is regulated by two promoters, namely the distal P1 promoter and the proximal P2 promoter (10).

AUTHOR CONTRIBUTIONS: Author 1: Kaviya Selvaraj carried out the study by collecting data and drafted the manuscript after performing the necessary statistical analysis and in the preparation of the manuscript.

Author 2: Padmapriya A aided in conception of the topic, designing the study and supervision of the study, correction and final approval of the manuscript.

Author 3: Dr. Senthilkumar Krishnamoorthi supervised the study design, analysis, and manuscript correction.

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5. CONCLUSION

In conclusion, the present study demonstrates for the first time that ethanolic extract of *C. halicacabum* facilitates the Runx2 mRNA expression in Osteoblast cells.

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