Original Article

# *In vitro* Molecular Mechanisms of Anticancer Activity of Stevioside in Human Osteosarcoma Cell Lines (Sarcoma Osteogenic)

**N. Prithiksha[1], R. Priyadharshini[2]**

*[1]Department of Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India, [2]Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India*

## Abstract

**Introduction:** Osteosarcoma (OS) is a rare and aggressive form of bone cancer that primarily affects the long bones of the body, such as the arms and legs. It is characterized by the uncontrolled growth of malignant cells in the bone tissue, leading to the formation of abnormal and painful bone masses. *Steviol* glycosides have been widely used as natural noncalorie sweeteners and are the collective name of the sweet substances found naturally in the plant *Stevia rebaudiana,* which is commonly called *Stevia*. Our study aimed to analyze the anticancer activity of *Stevioside* in OS. **Materials and Methods:** *Stevioside* was applied to OS cells, and the levels of Bcl xL, Bcl-2, and Bax were then estimated. The results of three separate studies, each carried out in triplicate, were expressed as the mean ± standard errors of the mean (SEM). One-way ANOVA was used for statistical analysis. **Results:** The findings showed that the effect of *Stevioside* on sarcoma osteogenic cells with mean ± SEM as 0.74 ± 0.05, 0.69 ± 0.09, 0.46 ± 0.09 for Bcl-xL gene, 0.98 ± 0.06, 0.58 ± 0.07, 0.5 ± 0.07 for Bcl-2 gene, and 1.2 ± 0.08, 1.45 ± 0.11, 1.67 ± 0.12 for Bax gene, respectively, when treated with untreated control cells. **Conclusion:** The study concludes its action against bone OS cells was significant with apoptotic induction. *Stevia* has a wide range of health benefits as well as being a plant-based diet it has less of side effects and promoting features even by intaking it daily along with other medicines.

**Keywords:** *Anticancer, cell line, innovation, osteosarcoma, Stevioside*

## Introduction

*Steviol* is a colonic metabolite of the natural sweetener *Steviol* glycosides. Currently, there are more than 150 species of *Stevia* (*Ocimum tenuiflorum*). *Stevia* is absorbed in the body and quickly modified in the liver. *Stevia* contains 11 major *Steviol* glycosides, of which rebaudioside A and *Stevioside* is the most abundant components in *Stevia*.[1] Body weight stores 4 mg of *Steviol*. Pure *Stevia* leaf extract can contain one *Steviol* glycoside or several different glycosides and is 250–300 times sweeter than sucrose. The *Stevia* leaves also contain several important phytochemical constituents such as alkaloids, chlorophyll, xanthophyll, oligosaccharides, amino acids, lipids, essential oils, free sugars, and hydroxycinnamic acids (caffeic acid).[2]

Osteosarcoma (OS) is a relatively rare tumor of bone with a worldwide incidence of 3.4 cases per million people per year. For most of the 20th century, 5-year survival rates of 56.31% for classic OS were very low.[3] In the 1970s, the introduction of adjuvant chemotherapy was a treatment for improving survival rates of OS. Conventional OS is the most common type and represents 80% of all OS cases, primarily affecting individuals in the first and second decades of life. It can be subdivided into osteoblastic, chondroblastic, and fibroblastic groups depending on the predominant features of the cells.[4] In the case of OS, the daily intake for *Stevia* leaf extract has been established and adopted globally in safety studies as 2 mg per pound of body weight expressed in *Steviol* equivalents.[5]

*Steviol* slowed the differentiation of OS cells. This might be due to the fact that *Stevia* extract has been theorized by many studies to have antioxidant properties. Antioxidants reduce the presence of free radicals from the body and since free radicals have the potential to damage DNA, they also contain the ability to disrupt gene expression in ways that increase abnormal differentiation of cancer cells.[6] *Steviol*



**How to cite this article:** Prithiksha N, Priyadharshini R. *In vitro* molecular mechanisms of anticancer activity of stevioside in human osteosarcoma cell lines (sarcoma osteogenic). Contemp Clin Dent 2024;15:198-201.

**Submitted :** 13-Sep-2023
**Revised :** 28-May-2024
**Accepted :** 08-Jul-2024
**Published :** 25-Sep-2024

***Address for correspondence:***
*Dr. R. Priyadharshini, Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 600 077, Tamil Nadu, India.*
*E-mail: priyadharshinir.sdc@saveetha.com*

**Access this article online**

**Website:** https://journals.lww.com/cocd

**DOI:** 10.4103/ccd.ccd_429_23

**Quick Response Code:**

inhibits bone cells through G1 phase cell cycle arrest, downregulating the ability of colony formation through mitochondrial apoptotic pathway, which was indicated by an increase of the Bax/Bcl-2 ratio and activation of cyclin-dependent kinase inhibitor 1, tumor protein 53, and cyclin-dependent kinase.[7]

Although previous studies on *Stevia* have been identified as an anticancer agent, its ability in inhibiting cell proliferation in OS has to be given in greater insight as its incidence has been noted and varied between 4% and 11% with increased mortality as well as to proceed the study with *in vivo* models. In this study, we investigated the anticancer effect of *Stevia* in sarcoma osteogenic (SaOs2) cell lines.

## Materials and Methods

The present study was done in the Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Chennai. The materials were collected from the National Center for Cell Science (NCCS), Pune, India.

### Chemicals

*Stevioside* was procured from sigma Aldrich. Trypsin-EDTA, fetal bovine serum (FBS), antibiotics-antimycotics, Dulbecco's modified Eagle's medium,and phosphate-buffered saline were purchased from Gibco, Canada. JC-1 (5,5,6,6-tetrachloro-1,1,3,3-tetraethyl benzimidazole carbocyanine iodide) and real-time PCR kit (MESA Green) were purchased from Invitrogen, USA. All the chemicals used were extra pure of analytical grade.

### Procurement and culture of sarcoma osteogenic cells

The OS cell line, SaOs2, was obtained from the NCCS, Pune, India, and cultured according to the cell culture instructions provided. Briefly, SaOs2 cells were grown in a minimal essential medium containing 10% FBS at 37°C in an atmosphere containing 5% $CO_2$.

### Cell viability assay

SaOs2 cancer cells were seeded at a density of 5 × 105 cells/well in 96-well plates and allowed to attach to the well overnight. After incubation, cultured cells were stimulated with various concentrations of 25 µM, 50 µM, and 100 µM *Stevioside* in triplicate and incubated at 37°C in a 5% humidified $CO_2$ incubator for 24 h. Subsequently, 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well, and incubation was continued for a further 4 h at 37°C. To dissolve the formazan formed from MTT, the cells were resuspended in 200 µL dimethyl sulfoxide, and the optical density (OD) of the solution was determined using a spectrometer at a wavelength of 570 nm. The experiments were repeated three times, independently. The mean OD ± standard errors of the mean (SEM) for each group of replicates was calculated. The entire procedure was repeated three times. The inhibitory rate of cell growth was calculated using the equation: % growth inhibition = (1-OD extract treated)/OD negative control ×100.

### Gene expression analysis by real-time polymerase chain reaction

Messenger RNA (mRNA) expression levels were examined using real-time polymerase chain reaction (PCR). The total RNA was isolated using Tri Reagent (Sigma). Total RNA (2 µg) from each sample was reverse transcribed using a commercial superscript III first-strand cDNA synthesis kit (Invitrogen, USA) according to the manufacturer's protocol. Real-time PCR was carried out in a MX3000p PCR system (Stratagene, Europe). The reaction was performed using MESA Green PCR master mix (it contains all the PCR components along with SYBR green dye.) Eurogentec, USA. The specificity of the amplification product was determined by melting curve analysis for each primer pair. The data were analyzed using the comparative computed tomography (CT) method and the fold change was calculated by the 2−CT method described by Schmittgen and Livak (2008) using CFX Manager Version 2.1 (Bio-Rad, USA).

### Statistical analysis

Data were expressed as the means ± SEM of three individual experiments performed in triplicate. Statistical analysis was performed using the one-way ANOVA, and $P < 0.05$ was considered to indicate a statistically significant result.

## Results

The anticancer activity of *Steviol* was assessed in the OS cell line (SaOs2). The study investigates the effect of *Steviol* on the viability of cells, where MTT assay was conducted in OS cell type [Figure 1]. The result demonstrates that *Steviol* induces their colony formation ability with a dose-dependent reduction in the viability of SaOs2 cells. *Steviol* exhibited a dose- and time-dependent inhibitory action on the proliferation of these cells, as it was demonstrated by a reduction in the viability of $SaO_{s2}$ cells.

In $SaO_{s2}$ cells, the effect of *Stevia* extract on the percentage of viable cells [Figure 2], Bcl-xL mRNA [Figure 3], BCL-2 mRNA [Figure 4], and Mcl-1 mRNA expression [Figure 5] were analyzed as mean values with their respective SEM for the Bcl-xL gene were 0.74 ± 0.05, 0.69 ± 0.09, and 0.46 ± 0.09. For the Bcl-2 gene, these values were 0.98 ± 0.06, 0.58 ± 0.07, and 0.5 ± 0.07. Finally, for the Bax gene, the mean values with their SEM were 1.2 ± 0.08, 1.45 ± 0.11, and 1.67 ± 0.12, respectively, when compared to untreated control cells.

## Discussion

The development of cancer involves a complex interplay among cellular processes, and a variety of cancer-promoting factors are involved**.** The mechanisms mediating

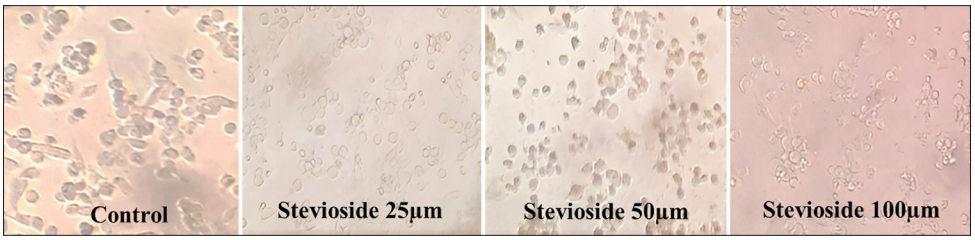


**Figure 1: *Stevioside* was treated with sarcoma osteogenic cell line (25 µM, 50 µM, and 100 µM) and morphological changes in cells were observed under an inverted light microscope at ×10 magnification**

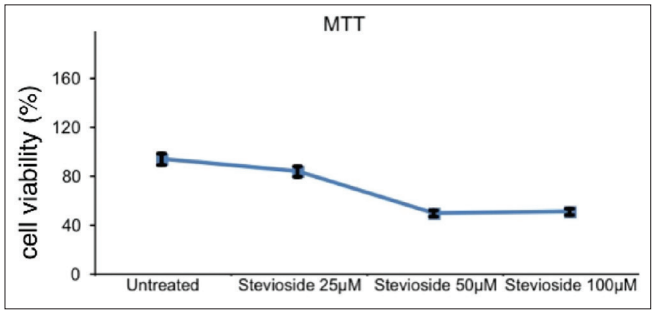


**Figure 2: Assessment of cell viability, in sarcoma osteogenic cells, the effect of *Stevia* extracts on cell viability with decrease in viable cells with rise in the concentration of *Stevioside* (µM)**

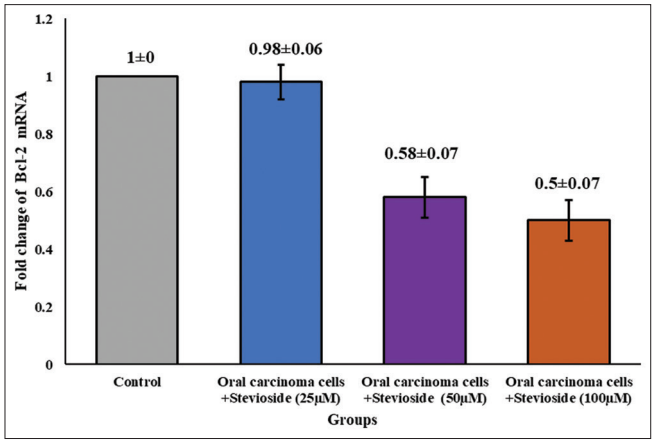


**Figure 4: Bar graph represents *Stevioside* and Bcl2 gene expression. The X-axis represents *Stevioside* concentration and the Y-axis represents the fold change over control. A mean ± standard deviation of observations is represented by each bar with significance at *P* < 0.05 with decrease in fold change of cancer cells with increased *Stevioside* (µM) concentration**

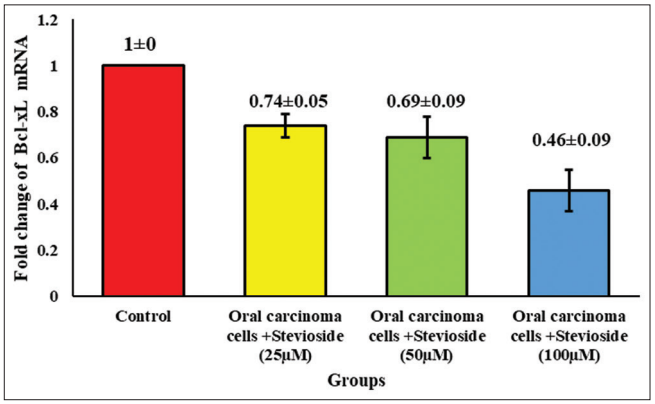


**Figure 3: Bar graph represents *Stevioside* and Bcl-xL gene expression. The X-axis represents *Stevioside* concentration and the Y-axis represents the fold change over control. A mean ± standard deviation of observations is represented by each bar with significance at *P* < 0.05 with decrease in fold change of cancer cells with increased *Stevioside* (µM) concentration**

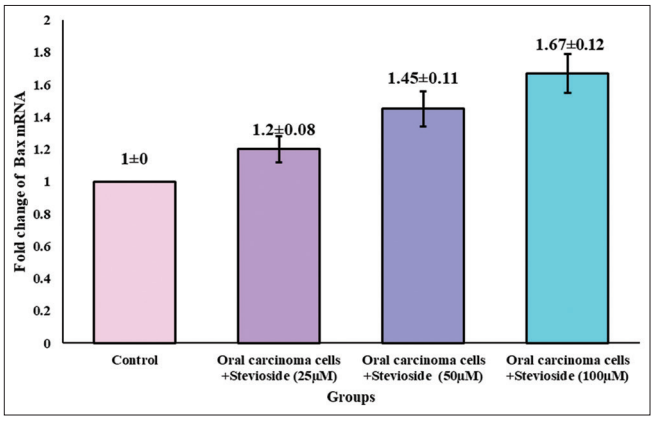


**Figure 5: Bar graph represents the *Stevioside* and Bax gene expression. The X-axis represents *Stevioside* concentration and the Y-axis represents the fold change over control. Increase in fold change of Bax gene with an increase in *Stevioside* (µM) concentration**

*Stevia* include mainly cell cycle arrest, through the regulation of key proliferation proteins, and induction of the apoptotic process. Our study results revealed a decrease in the number of viable cancer cells with decreased fold change of antiapoptotic genes and increase in proapoptotic fold change favoring as an anticancer agent. Phytochemical compounds enact its role as an antioxidant by inducing apoptosis and decreasing cell proliferation.[8]

A previous study of anticancer activity of *Stevioside* with a lethal level (IC50) of 185 µM in a human breast cancer cell line (MCF-7) indicated significance with G2/M phase arrest along with G0/G1 peak showing the presence of apoptosis taking place.[9] Studies on *Stevia's* scavenging activity along with its phenolic acid compounds toward free radicals, came up with lethality at 51.98 mg/mL, disclosing its antioxidant property prevented malignant cell lines from proliferating.[10] *Stevioside* treatment yielded IC50 values of 55 µM and 66 µM for the cancer cells MDA-MB-231 and SKBR3, respectively.[11] Research demonstrates that pure *Stevioside* can trigger apoptosis, indicating the substance has potential anticancer properties. Furthermore, flavonoids in the leaves of *Stevia* was found to have hydroxyl, superoxide, and free radical scavenging effects.[12]

*Stevioside* enhances insulin secretion and improves insulin sensitivity, leading to lower blood glucose levels. Studies in both diabetic and nondiabetic subjects have demonstrated that *Stevioside* can reduce postprandial glucose levels, which is potentially beneficial for managing diabetes.[13] It promotes the relaxation of blood vessels, which can reduce blood pressure.[14] This effect is particularly beneficial for hypertensive individuals.[13] *Stevioside* helps to protect cells from oxidative damage, which is linked to various chronic diseases, including cardiovascular diseases and cancer.[15]

Even though these cell line model systems are very useful for initial screening, drug response phenotypes using cell line model systems still need to be confirmed by functional validation and mechanistic studies, as well as validation studies using clinical samples. The study primarily uses the $SaOs_2$ OS cell line to assess the anticancer activity of *Steviol*. While cell line models are valuable for preliminary screening, they do not fully replicate the complexity of human tumors. The results observed *in vitro* may not accurately predict *in vivo* responses. The research is limited to a single type of cancer cell line (OS). This restriction may not provide a comprehensive understanding of *Steviol's* anticancer efficacy across different types of cancer. Future studies should explore the anticancer activity of *Steviol* across a variety of cancer cell lines, including those from different tissues and genetic backgrounds, to determine the broader applicability of the findings.

## Conclusion

The study concludes its action against bone OS cells was significant with apoptotic induction. As an added advantage, natural additive sweeteners like *Stevia* have a wide range of health benefits; as well as being a plant-based diet, it has less of side effects and more of safer health-promoting features even by intaking it daily along with other conventional medicines.

### Acknowledgment

We thank Saveetha Dental College and Hospitals for the successful completion of the study.

### Financial support and sponsorship

The present project is funded by
- Saveetha Institute of Medical and Technical Sciences
- Saveetha Dental College and Hospitals and Saveetha University
- Saveetha University
- Jyothi Gas Agency.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Iatridis N, Kougioumtzi A, Vlataki K, Papadaki S, Magklara A. Anti-cancer properties of *Stevia rebaudiana*; more than a sweetener. Molecules 2022;27:1362.
2. Wu D, Wang Z, Lin M, Shang Y, Wang F, Zhou J, *et al. In vitro* and *in vivo* antitumor activity of cucurbitacin C, a novel natural product from cucumber. Front Pharmacol 2019;10:1287.
3. Yasin NF, Abdul Rashid ML, Ajit Singh V. Survival analysis of osteosarcoma patients: A 15-year experience. J Orthop Surg (Hong Kong) 2020;28:2309499019896662.
4. Zhao X, Wu Q, Gong X, Liu J, Ma Y. Osteosarcoma: A review of current and future therapeutic approaches. Biomed Eng Online 2021;20:24.
5. Nandita R, Priyadharshini R, Rajeshkumar S, Sinduja P. Cytotoxic effect of *Pterocarpus santalinus* and *Stevia*-based mouthwash – A lab-based analysis. J Pharm Res Int 2021;33:437-47.
6. Priyadharshini R, Kumar SR, Sinduja P. Herbal formulation of *Azadirachta indica* and *Stevia rebaudiana* and its anti-inflammatory and anti-diabetic activity. J Pharm Res Int 2021;33:200-7.
7. Ramya G, Varghese SS, Rajeshkumar S. Anti inflammatory and antioxidant activity of *Stevia* and peppermint herbal formulation. J Pharm Res Int 2021;33:10.
8. Mutmainah, Kusmita L, Martono Y, Franyoto YD, Wulandari RP, Kusumaningrum TD. Antioxidant activity, phenol and flavonoid content, and formulation cream of *Stevia rebaudiana* Bert. J Phys Conf Ser 2019;1217:012152.
9. Gupta E, Kaushik S, Purwar S, Sharma R, Balapure AK, Sundaram S. Anticancer potential of steviol in MCF-7 human breast cancer cells. Pharmacogn Mag 2017;13:345-50.
10. Ruiz-Ruiz JC, Moguel-Ordoñez YB, Matus-Basto AJ, Segura-Campos MR. Antidiabetic and antioxidant activity of *Stevia rebaudiana* extracts (Var. Morita) and their incorporation into a potential functional bread. J Food Sci Technol 2015;52:7894-903.
11. Khare N, Chandra S. Stevioside mediated chemosensitization studies and cytotoxicity assay on breast cancer cell lines MDA-MB-231 and SKBR3. Saudi J Biol Sci 2019;26:1596-601.
12. Myint KZ, Wu K, Xia Y, Fan Y, Shen J, Zhang P, *et al.* Polyphenols from *Stevia rebaudiana* (Bertoni) leaves and their functional properties. J Food Sci 2020;85:240-8.
13. Nivedha VM, Priyadarshini R, Rajeshkumar S, Sinduja P. Anti-diabetic and antioxidant activity of *Pterocarpus santalinus* and *Stevia* herbal formulation. J Pharm Res Int 2021;33:11.
14. Al-Dujaili E, Twaij H, Bataineh YA, Arshad U, Amjid F. Effect of *Stevia* consumption on blood pressure, stress hormone levels and anthropometrical parameters in healthy persons. Am J Pharmacol Toxicol 2017;12:7-17.
15. Sinduja P, Rajeshkumar S, Priyadharshini R, Selvapriya S. Anti-inflammatory and cytotoxic effect of *Stevia* and Neem based herbal formulation. J Pharm Res Int 2021;33:150-60.