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Exploring the Molecular Mechanisms of Stevioside's Chemopreventive Anticancer Effects on Human Prostate Cancer Cells In Vitro

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Abstract

The global incidence and mortality rates of cancer are rapidly escalating, with prostate cancer ranking as the second most prevalent form of cancer in men, affecting approximately 1.41 million people worldwide (according to data from the World Health Organization (WHO)). Stevioside, a naturally occurring compound, has shown promising results in inhibiting the growth of cancer cells. This research seeks to investigate the molecular pathways responsible for the chemopreventive anticancer effects of Stevioside in prostate cancer cell lines. Prostate cancer cells were exposed to Stevioside, and the expression levels of Bcl-2, Mcl-1, and caspase-3 were measured. The findings, presented as the mean ± standard deviation from three separate experiments, were analyzed using one-way ANOVA. In PC-3 cells, Stevioside treatment significantly reduced cell viability, as indicated by the MTT assay, and resulted in a decrease in Mcl-1 gene expression with increasing concentrations. In addition, a significant increase in caspase-3 expression suggested enhanced apoptosis, while Bcl-2 levels also declined with higher concentrations, demonstrating statistical significance ($P < 0.05$). The results indicate that Stevioside significantly inhibits the growth of prostate cancer cells, positioning it as a potential natural therapeutic option. Further research is needed to determine the safe and effective daily intake of Stevia-based products for human use.

Keywords: Cancer prevention, Prostate cancer, Stevioside, Natural therapy, Cell apoptosis, Health

Introduction

Prostate cancer is one of the most commonly diagnosed invasive cancers. According to 2022 statistics, there were 112 new cases per 100,000 men annually, with an increasing mortality rate of 18 per 100,000 men [1]. The standard treatment approach for prostate cancer is androgen deprivation therapy, which typically provides only a temporary response [2]. Additionally, chemotherapy has been used to help manage the disease, but it does not always offer long-term success. Inducing apoptosis in tumor cells is a promising strategy to prevent cancer progression, and a variety of natural, readily available substances can be used as therapeutic agents to prevent further cancer cell invasion and proliferation [3]. Natural sources offer a rich variety of structural frameworks that can lead to the discovery of effective chemical agents for treating prostate cancer [4]. Stevioside, a naturally occurring compound, has been shown to inhibit the growth of cancer cells significantly. It also supports urinary health and possesses a range of beneficial properties, including hypotensive, vasodilating, antifungal, antiviral [5], anti-inflammatory, anti-diabetic [6], antibacterial, and antioxidant effects [7]. Research indicates that Stevioside, derived from

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plants grown in Brazil, Japan, Paraguay, and other regions, is non-toxic, non-addictive, and free from carcinogenic or mutagenic effects. It does not alter blood sugar levels, making it safe for diabetic patients. Despite these benefits, the challenge of treatment resistance and the risk of cancer recurrence have prompted scientists to explore new anticancer agents, particularly those derived from medicinal plants. *Stevia rebaudiana*, a plant native to Paraguay and Brazil, produces Stevioside in its leaves and has long been used for its medicinal properties [8, 9]. While approximately 70 species of *Stevia* are found in Mexico, research on their anticancer effects remains limited. These species contain bioactive compounds such as flavonoids, sterols, and sesquiterpenes, which are believed to contribute to their therapeutic potential [10]. This study aims to investigate the molecular mechanisms through which Stevioside exerts chemopreventive anticancer effects in human prostate cancer cell lines.

Materials and Methods

Chemicals

Stevioside was procured from Sigma-Aldrich. Cell culture reagents including trypsin-EDTA, fetal bovine serum (FBS), antibiotics, antimycotics, Dulbecco's Modified Eagle's Medium (DMEM), and phosphate-buffered saline (PBS) were acquired from Gibco (Canada). Real-time PCR kits and JC-1 were sourced from Invitrogen (USA). All chemicals used were of the highest available quality and purity.

Cell line and culturing conditions

The human prostate cancer cell line was supplied by the National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in MEM supplemented with 10% FBS and maintained in a CO2 incubator set at 37 °C and 5% CO2.

Cell viability assay

For the viability assay, prostate cancer cells were seeded in 96-well plates at 5 x 10^5 cells per well and allowed to adhere overnight. They were then treated with varying concentrations of Stevioside for 24 hours. To assess cell viability, an MTT assay was performed. After 4 hours of incubation with MTT, the formazan crystals were dissolved in DMSO, and absorbance at 570 nm was measured with a spectrophotometer. The percentage of growth inhibition was calculated using the formula:

$$\text{Growth inhibition (\%)} = [(\text{OD control} - \text{OD treated}) / \text{OD control}] \times 100 \quad (1)$$

Gene expression analysis via Real-Time PCR

RNA extraction was performed using tri reagent (Sigma), and 2 µg of RNA was reverse transcribed into cDNA using the superscript III first-strand synthesis kit (Invitrogen). Real-time PCR was carried out with the MESA green PCR master mix (Eurogentec, USA), which includes SYBR Green dye. Primer specificity was confirmed by melting curve analysis. The resulting data were analyzed using the comparative CT method, and fold changes in gene expression were calculated using the 2^-ΔΔCT method and analyzed with CFX Manager (Bio-Rad, USA).

Statistical analysis

Each experiment was performed three times, with all assays conducted in triplicate. The results were expressed as means ± standard deviations. Statistical significance was determined using one-way ANOVA, and a P-value of less than 0.05 was considered statistically significant.

Results and Discussion

In the PC-3 prostate cancer cell line, the effects of Stevioside on cell viability were assessed using the MTT assay **(Figure 1)**. The results demonstrated a significant reduction in the percentage of viable cells as the Stevioside concentration increased. Furthermore, the expression of the Mcl-1 gene **(Figure 2)** showed a concentration-dependent decrease. Caspase 3 expression **(Figure 3)** notably increased with higher concentrations of Stevioside, suggesting that apoptosis was being effectively induced. Additionally, Stevioside inhibited the expression of the Bcl-2 gene **(Figure 4)**, with a significant reduction in fold change at higher concentrations. Statistical significance ($P < 0.05$) was confirmed using one-way ANOVA, supporting the conclusion that Stevioside possesses strong anticancer properties.

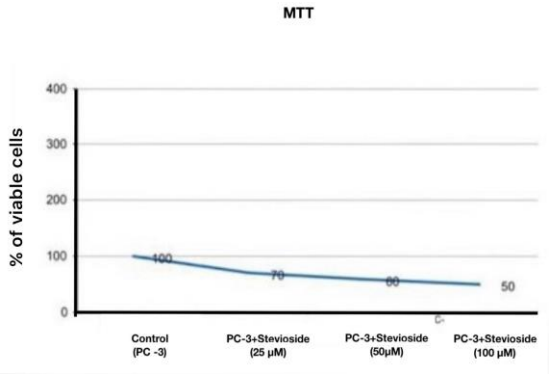

Figure 1. Cell viability analysis using the MTT assay.

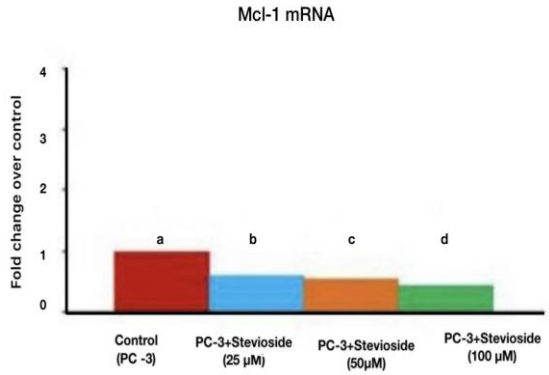

Figure 2. Effect of Stevioside on Mcl-1 gene expression; the graph plots Stevioside concentrations (a) control (Red), (b) 25 µM (blue), (c) 50 µM (orange), and (d) 100 µM (green) on the X-axis, with the Y-axis showing the fold change in gene expression relative to control; at higher concentrations (100 µM), Stevioside significantly reduced Mcl-1 expression.

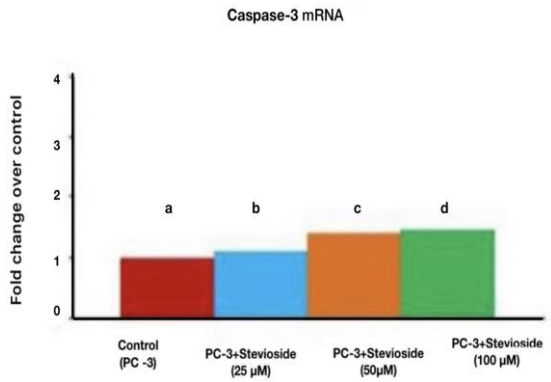

Figure 3. Effect of Stevioside on caspase 3 gene expression; the X-axis indicates different Stevioside concentrations (a) control (red), (b) 25 µM (blue), (c) 50 µM (orange), and (d) 100 µM (green), while the Y-axis shows the fold change over control; an increase in caspase 3 expression was observed with higher concentrations of Stevioside, indicating enhanced apoptosis.

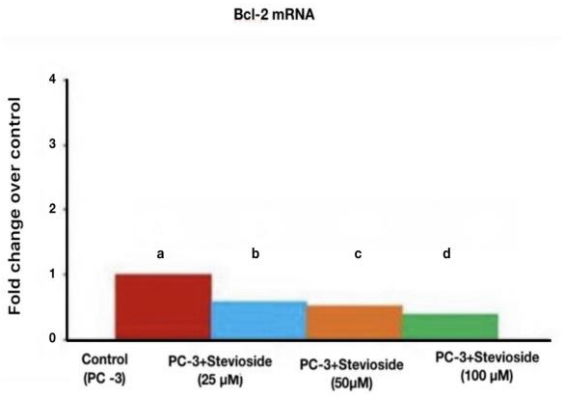

Figure 4. Effect of Stevioside on Bcl-2 gene expression; the X-axis represents Stevioside concentrations (control: red, 25 µM-blue, 50 µM-orange, and 100 µM-green), and the Y-axis shows fold change over control; a significant reduction in Bcl-2 expression was observed at higher Stevioside concentrations, suggesting inhibition of anti-apoptotic pathways.

These results suggest that Stevioside exerts a significant anticancer effect on prostate cancer cells, primarily by modulating key genes involved in apoptosis. Further research is necessary to confirm these findings in vivo and explore the potential therapeutic applications of Stevioside for prostate cancer treatment.

In this study, the anticancer properties of Stevioside were assessed using an in vitro human prostate cancer cell line. The results showed a positive correlation between Stevioside treatment and the activation of caspase 3, as well as a negative correlation with the expression of Mcl-1 and Bcl-2 genes, both of which are involved in regulating apoptosis. Previous studies on castration-resistant prostate cancer (CRPC) demonstrated that chemotherapeutic agents targeting Mcl-1 mRNA could trigger cell death in response to DNA damage, further supporting the role of Mcl-1 in cancer progression and treatment resistance [11].

In this study, increasing Stevioside concentration led to a marked reduction in Bcl-2 mRNA expression when compared to the control group. This is consistent with previous findings that Stevioside induces apoptosis in

cancer cells, making it a promising candidate for anticancer therapy [12]. Notably, prostate cancer cells treated with Stevioside did not exhibit significant negative side effects, suggesting its potential for therapeutic use without the typical toxicities associated with conventional chemotherapy [13, 14].

The upregulation of caspase-3 gene expression with increasing Stevioside concentrations further supports its role in promoting apoptosis. This finding aligns with previous studies where Stevioside was shown to enhance the apoptotic effects when used in conjunction with silver nanoparticles, demonstrating its stability and effectiveness in other cancer types, such as hepatocellular carcinoma [15].

Despite the promising results, it is important to note that Stevioside's biological and pharmacological properties are still being studied, and there are gaps in knowledge that prevent it from being granted "generally recognized as safe" (GRAS) status by the food and drug administration (FDA) [16]. While Stevioside is approved as a food additive in several countries, its use in the U.S. remains restricted to dietary supplements. Further studies are needed to understand its full pharmacological potential and to determine safe, effective dosages for human use.

Future research should expand upon these findings by testing Stevioside in a variety of cell lines and exploring additional gene targets to enhance its anticancer efficacy. Additionally, research could explore the long-term effects of Stevioside as a potential therapeutic for prostate cancer and other malignancies.

Conclusion

This study provides strong evidence that Stevioside has significant anticancer effects in prostate cancer cells, primarily by modulating apoptotic pathways. The findings suggest that Stevioside could be developed as a natural therapeutic for prostate cancer. However, further research is essential to establish safe and beneficial dosages for regular consumption, and to explore the broader therapeutic potential of Stevioside for cancer treatment.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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