ORIGINAL RESEARCH

Chemotherapeutic Effect of Rutin on TGF-β/SMAD2 Signalling Molecules Gene Expression in Oral Cancer Cells.

Srimathi B[1], Anitha Roy[1], Perumal Elumalai[2]

Abstract

Introduction: The flavonoid rutin, which is frequently present in a variety of vegetables and fruits, has drawn interest because of its wide range of biological actions, which include anti-inflammatory, anticancerand antioxidant capabilities. According to recent research, rutin shows promise as a chemotherapy treatment for a variety of cancers, including oral cancer. Oral cancer formation and progression are linked to dysregulation of TGF-β signalling pathway components, including SMAD2 molecules. The multifunctional cytokine known as transforming growth factor-beta, or TGF-β, is essential for controlling immunological responses, cell development, and differentiation.

Aim: The aim of the present study was to evaluate the anticancer property of Rutin and its effect on TGF-β/SMAD2 pro-tumorigenic signals.

Materials and Methods: The MTT assay was used to evaluate the cell viability of rutin-treated oral cancer cells.Using a phase contrast microscope, cell morphology was examined. Real-time PCR was used to analyse the TGF-β/SMAD2 gene expression.

Results: Rutin demonstrated a strong cytotoxic and apoptotic potency against the oral cancer cell line in the MTT assay, and this was confirmed by notable morphological changes observed under a phase contrast microscope during a 24-hour treatment period. The findings of the cell viability assay demonstrated that rutin-treated (KB-1) cells exhibited 50% growth inhibition at a concentration of 43.8µM/ml. This concentration was determined to be the inhibitory concentration (IC-50) dose value and was set aside for future research. The TGF-β/SMAD2 gene expression signalling pathway was suppressed by rutin.

Conclusion: This study concludes that rutin can protect against oral cancer as it can modulate the TGF-β/SMAD2 signaling pathways and may help to reduce tumour growth overall. Rutin may also suppress SMAD2 phosphorylation, which would interfere with SMAD2's phosphorylation-dependent activation and the subsequent TGF-β pathway signaling events. Hence, rutin can be considered as a useful candidate for the treatment of oral cancer.

Keywords: Cancer therapy, oral cancer, Phytochemicals, Rutin, TGF-β/SMAD2,

Introduction

Mouth cancer, sometimes referred to as oral cavity cancer or oral cancer, is a malignancy that occurs in the tissues of the mouth, including the lips, tongue, cheeks, gums and throat[1]. It is a serious and potentially life-threatening disease characterized by the uncontrolled growth of abnormal cells within the oral cavity[2]. These cancer cells typically result from genetic mutations or other factors that disrupt the normal cell cycle, causing them to divide and grow uncontrollably. Oral cancer cells can lead to the formation of tumors within the mouth, and if left untreated, they can spread to other parts of the body, making early detection and treatment critical for a favourable prognosis[3]. Various types of oral cancer cells may exist, each with its own characteristics and behavior, which can influence treatment decisions and outcomes.

Surgery, radiation, photodynamic therapy, stem cell therapy, and chemo/immunotherapies are common cancer treatment modalities. Chemotherapy is an effective treatment,

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but its clinical application is hindered by the drawbacks of traditional cytotoxic treatments, such as severe side effects, high costs, and multiple drug resistance[4]. To get the best outcomes, it is therefore imperative to find novel, safe, and effective therapeutic choices. Natural compounds generated from plants have drawn a lot of interest in medication development initiatives. Natural sources have provided a number of medications used in cancer treatment, such as vinblastine, paclitaxel, camptothecin, and doxorubicin[5]. Natural chemicals are very interesting to avoid related adverse effects in cancer treatment since they are possible multi-targeted agents in the fight against cancer[5,6]. It is becoming evident that the cytostatic actions of natural products result from their capacity to control oxidative stress, inflammation, autophagy, and apoptosis, hence preventing or reducing the toxicity. Understanding the intricate balance between the tumor-suppressive and pro-tumorigenic roles of TGF-β/SMAD2 signalling is crucial[6]. Rutin, by influencing this pathway, has the potential to modulate gene expression and restore a more balanced signaling environment, providing a targeted approach for oral cancer treatment[7].

Flavonol glycoside rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside) is present in a broad range of foods and drinks, such as passionflower, grapes, apples, tea, and wine. Rutin's antioxidant properties may help counteract oxidative stress, a common factor in oral cancer development. By scavenging free radicals, Rutin could contribute to reducing oxidative damage to cellular components. Chronic inflammation is linked to cancer progression, and Rutin's anti-inflammatory properties may help mitigate this process. By modulating inflammatory pathways, rutin could potentially inhibit inflammatory signals associated with oral cancer. It has been documented that rutin causes cancer cells to undergo apoptosis, or programmed cell death[8]. This mechanism is crucial for controlling abnormal cell growth, and Rutin's ability to trigger apoptosis could be relevant in combating oral cancer. Rutin has demonstrated anti-angiogenic effects, meaning it may hinder the formation of blood vessels that supply nutrients to tumors. In oral cancer, inhibiting angiogenesis can be a strategy to impede tumor growth and metastasis[9]. Rutin has been studied for its influence on various signalling pathways, including those involved in cancer progression. In the context of oral cancer, Rutin might affect pathways such as TGF-β/SMAD2, influencing gene expression and potentially disrupting the signals that drive cancer growth. Given the role of TGF-β/SMAD2 signaling in cancer progression, flavonoids have ability to interfere with this pathway may have anticancer effects[10]. Rutin may interfere with the TGF-β/SMAD2 pro-tumorigenic signals, which would decrease the growth and spread of cancer cells. Therefore the aim of the present study was to evaluate the anticancer property of rutin and its effect on TGF-β/SMAD2 pro-tumorigenic signals.

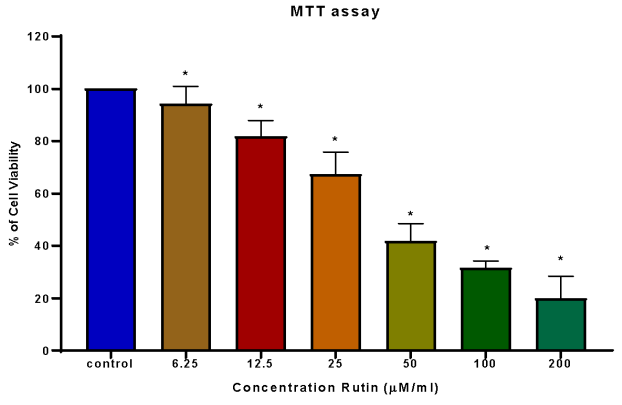


Fig.1: The cytotoxic effects of Rutin on oral cancer cells. Cells were treated with extract Rutin (6.25- 200 µM/ml) for 24h, and cell viability was evaluated by MTT assay. Data are shown as means ± SD (n = 3). * Compared with the control blank group, $p < 0.05$.

Materials and Methods

Rutin was obtained from LOBACHEMIE PVT. LTD, Mumbai, India with Cas No.3420-18-4 MSDS product code 05621. The NCCS in Pune provided the oral cancer cell lines (KB-1). The cells were cultured in T25 culture flasks with DMEM that had been enhanced with 1% antibiotics and 10% FBS. The cells were kept in a humidified environment with five percent CO_2 at 37^0C. The cells were trypsinized and passaged once they reached confluency.

Cell viability (MTT) Assay

The MTT assay was used to evaluate the cell viability of rutin-treated oral cancer cells[11]. The assay is based on the conversion of soluble yellow tetrazolium salt by metabolically active cells to insoluble purple formazan crystals. At a density of $5x10^3$ cells/well, KB-1 cells were plated in 96-well plates. Following plating, the cells were starved for three hours at 37^0C by incubating them in serum-free media and then washing them twice with 100µl of the medium. Following fasting, cells were exposed to rutin at various concentrations (6.25- 200µM/ml) for a whole day. After the treatment was completed, each well received 100µl of MTT containing DMEM (0.5 mg/ml), and the media from the treated and control cells was discarded. After that, the cells were cultured inthe CO_2 incubator for 4 hours at 37^0C. After discarding the media containing MTT, the cells were cleaned using one PBS wash. Following the formation of formazan crystals, 100µl of dimethyl sulfoxide was added and the mixture was left in the dark for an hour. Next, a Micro ELISA plate reader set to 570 nm was used to measure the colorintensity developed. As a proportion of control cells grown in serum-free media, the quantity of viable cells was reported. In the control media, there was no treatment, and cell viability was 100%. The formula for calculating cell viability is as follows: % cell viability = [A570 nm of treated cells/A570 nm of control cells]×100.

Morphology study

We determined the ideal dose (IC-50: 43.8µM/ml) for additional research based on the MTT experiment. Examination of morphological changes in cells using a phase contrast microscope. After being planted in six well plates, 2×105 cells were exposed to rutin for a whole day. The media was removed and the cells were once again rinsed with phosphate buffer

saline (PBS pH 7.4) at the conclusion of the incubation time. A phase contrast microscope was used to examine the plates.

Real Time PCR

Real-time PCR was used to analyze the TGF β/SMAD2 signalling molecule's gene expression. Using TrizolReagent (Sigma) and a defined technique, total RNA was extracted. Using a PrimeScript, first strand cDNA synthesis kit (TakaRa, Japan), 2μg of RNA were utilized for reverse transcription-based cDNA synthesis. Particular primers were used to amplify the targeted genes. GoTaq® qPCR Master Mix (Promega), which includes all of the PCR components and SYBR green dye, was used to run the PCR reaction. Utilizing a Biorad CFX96 PCR machine, real-time PCR was carried out. The comparative CT approach was utilized to assess the results, and Schmittgen and Livak's 2−ΔΔCT method was employed to calculate the fold change.

Statistical analysis

Using SPSS, a One-way ANOVA proceeded by Students-t-test was used to examine all of the data, which were expressed as mean ± SD for triplicates. $P < 0.05$ was considered as statistically significant..

Results

Effect of rutin on cell viability of oral cancer cell line

The MTT assay was used to evaluate rutin's cytotoxic capability against oral cancer cells. A full day, the cells were exposed to rutin at varying doses (6.25-200μM/ml). At 24-hour mark, rutin therapy dramatically reduced the vitality of KB-1 cancer cells when compared to the control (Figure 1). As the concentration increased, the fraction of viable cells decreased progressively 50% growth inhibition was seen at 43.8μM/ml of concentration. IC-50 dose (43.8μM/ml) was therefore taken into consideration for more research.

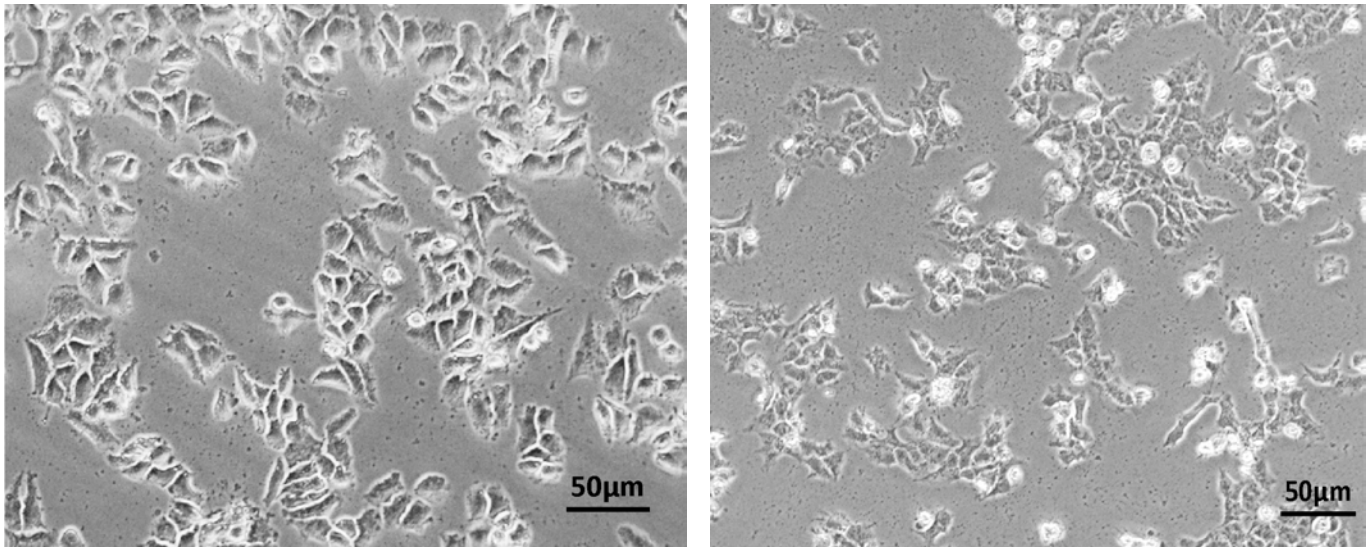
The effect of rutin on cell morphology

Using an inverted phase contrast microscope, the morphological examination of oral cancer cells treated with rutin was conducted. After subjecting the KB-1 cells to a 24-hour rutin treatment (43.8μM/ml), considerable morphological changes, typical of apoptotic cells, were detected in the treated cells (Figure 2). These alterations included cell shrinkage and reduced cell density. Other morphological alterations, like rounded cells that shrink and lose contact with nearby cells, were also seen in apoptotic cells. Even some delicate cells separated from the plate surface.

The graph shows how the oral cancer cell line changed morphologically both with and without rutin treatment. Following a 24-hour rutin treatment (43.8μM/ml), the cells were examined using an inverted phase contrast microscope. Following rutin (43.8μM/ml) treatment, the number of cells dropped and they exhibited notable morphological changes, such as cell shrinkage and cytoplasmic membrane blebbing, that are indicative of apoptotic cells.

The effect of rutin on TGF-β/SMAD2 gene expression

Figure 3 displays the fold change for the TGF- β /SMAD 2 gene expression. A down regulation of the genes was demonstrated by the fold change, suggesting good anti-cancer potential. The target gene expression is expressed as a fold change from the control after being normalized to the expression of GAPDH mRNA. Each bar shows the three independent observations' mean plus standard deviation. At the $p<0.05$ level, the symbol "*" denotes statistical significance between the drug treatment and control groups.



(a Control) (b.Rutin (43.8 μM/m

Fig. 2: Effect of Rutinon cell morphology of human oral cancer cell line (KB-1) at 20X magnification. Cells were treated with rutin(43.8μM/ml)for 24 h and cells were observed under inverted phase contrast microscope. The number of cells decreased after rutin (43.8μM/ml) treatment and the cells exhibited cell shrinkage and cytoplasmic membrane blebbing.

Discussion

For dental surgeons in particular, oral cancer is an extremely important global public health issue. Oral cavity or lip can develop malignant neoplasia, which is known as oral cancer. consists of oral squamous cell carcinoma (OSCC), which is the term used traditionally since 90% of malignancies in the dental area have squamous cell origins[12]. A concerning health issue, oral and oropharyngeal cancers are among the most prevalent cancers in the world. Treatment options include surgery, chemotherapy and radiation[13]. However, these treatment options on long-termcause morbidities, severe acute toxicities and swallowing dysfunctions and thus need for replacement or improvement[14].

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a bioactive flavonoid that belongs to the kingdom of plants. Rutin has been studied as a potential anticancer medication due to its numerous pharmacological properties, which include antioxidative, anti-inflammatory, and anticancer actions[15]. Rutin has been found to affect a wide range of molecular targets implicated in the development of cancer, such as transcription factors, drug transporters, inflammatory cytokines, cellular kinases, and mediators of the cell cycle[16].

The MTT assay is a widely used calorimetric technique to assess the vitality and proliferation of cells. This test is widely used in cell biology and drug development research to assess the effects of chemicals on cell viability[11,17]. In this experiment, the concentration rose with a progressive decrease in the percentage of viable cells (Figure 1). 50% growth inhibition was seen at 43.8µM/ml of dose. Therefore, the IC 50 dose (43.8µM/ml) was considered for further investigation.

Analysing and studying the forms and physical attributes of cells is done through the use of cell morphology test methods. When it comes to cellular operations, health, and reactions to different situations, these assays offer important insights into the composition, dimensions, and outward look of cells. Under a phase contrast microscope, the KB-1 cell line was examined following rutin therapy. Following the application of rutin (43.8µM/ml), the cells showed signs of blebbing of the cytoplasmic membrane and cells reduced in size and number (Figure 2). This shows rutin supports the cancer cell death by having strong proapoptotic effects.

The TGF- β /SMAD2 signalling pathway involves the phosphorylation of SMAD2, which causes it to translocate to the nucleus and regulate target gene transcription. TGF- β / SMAD2 signaling has several roles, including influencing target genes linked to extracellular matrix formation, cell cycle regulation, death, and immune response. The influence of rutin on TGF-ß/SMAD2 gene expression displays the fold change in TGF β /SMAD2 gene expression. Strong anti-cancer potential was indicated by the down regulated genes, as indicated by the fold change (Figure 3). The findings of a study showing that rutin causes apoptosis in KB-1 and inhibits the TGF β / SMAD2 signaling pathway. The findings imply that rutin causes apoptosis in KB-1 cells via suppressing the TGF- β and SMAD2 gene expression signalling pathway. Thus, rutin can be utilized as a potent, natural anticancer medication. Many other plants extracts are also biologically explored for their anticancer activity like `rutin[18,19,20].

Conclusion

Based on the modulation of the TGF-β/SMAD2 signalling pathways, rutin protects against oral cancer, according to these findings. Rutin may help reduce tumour growth overall by inhibiting TGF-β/SMAD2 signalling, which in turn limits the ability of oral cancer cells to proliferate. Rutin may also

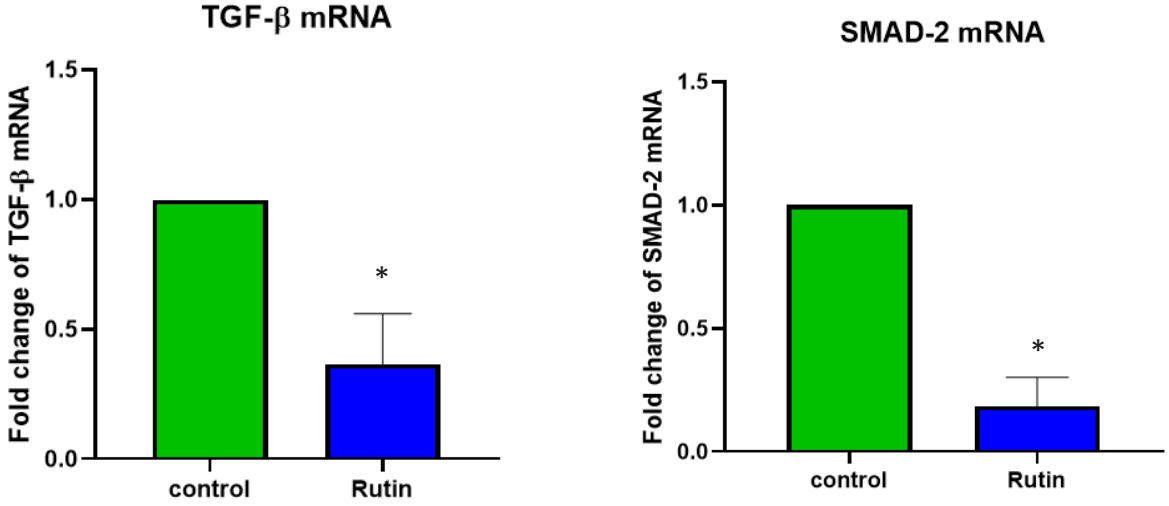


Fig. 3: Rutin (43.8µM/ml) effects the expression of the TGF-β/SMAD-2 genes in oral cancer cell lines. The target gene expression is expressed as a fold change from the control after being normalized to the expression of GAPDH mRNA. Each bar shows three independent observations' mean plus standard deviation. At the $p<0.05$ level, the symbol "*" denotes statistical significance between the drug treatment and control groups.

suppress SMAD2 phosphorylation, which would interfere with SMAD2's phosphorylation-dependent activation and the subsequent TGF-β pathway signalling events. Rutin may therefore be useful in the treatment of oral cancer.

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