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Evaluation of Anticarcinogenic Potential of Emodin (Aloe vera Extract) on Tongue Squamous Cell Carcinoma Cell Line

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ORIGINAL STUDY

Evaluation of Anticarcinogenic Potential of Emodin (Aloe Vera Extract) on Tongue Squamous Cell Carcinoma Cell Line

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Abstract

Purpose: This study was to assess the potential anticarcinogenic effect of emodin (aloe vera extract) on a tongue squamous cell carcinoma cell line (SCC25). **Patients and methods:** Six groups were included in this study; groups I and II: different concentrations of emodin and 5-fluorouracil (5-FU), respectively, were applied to the WI38 normal epithelial cell line. The 3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide cytotoxicity test was performed to explore the cytotoxicity of both substances on WI38 normal epithelial cells. Groups III to VI: Various concentrations of emodin, 5-FU, and a combination of both substances at different ratios, respectively, were applied to the cultivated SCC25 cell line to choose the concentrations with high cytotoxic effects according to their IC50 values at 48 and 72 h. **Results:** The IC50 of group I emodin/WI38 (106 ± 4.93) and (93.4 ± 3.56) was higher than that of group II 5-FU/WI38 (49.0 ± 2.29) and (45.8 ± 1.48) at 48 and 72 h, respectively, meaning that lower concentration of 5-FU was more cytotoxic to normal cells than emodin. While the highest IC50 mean values were in group III emodin/SCC25 (47.0 ± 2.28) and (38.6 ± 3.56), then group IV 5-FU/SCC25 (12.50 ± 0.6) and (7.62 ± 0.29), followed by group VI Emodin + low 5-FU/SCC25 (5.41 ± 0.26) and (6.91 ± 0.26), with the least value being in group V Emodin + high 5-FU/SCC25 (3.29 ± 0.16) and (2.89 ± 0.11) at 48 and 72 h, respectively, meaning that addition of emodin to either high or low concentrations of 5-FU will decrease the concentration than if 5-FU or emodin used alone. **Conclusion:** Adding emodin to either high or low concentrations of 5-FU lowers the concentration compared with using 5-FU or emodin alone.

Keywords: Anticarcinogenic, Cell line, Emodin

1. Introduction

The tongue is the most common site of oral cancer. One of the most lethal head and neck cancers is tongue squamous cell carcinoma (TSCC). It is silent and progresses from a premalignant state to invasive carcinoma without any symptoms. This causes delays in diagnosis and leads to poor prognosis [1]. TSCC is characterized by aggressive biological behaviour, with a high incidence rate of lymph node and remote metastasis. Although the 5-year survival rate is reported to be up to 50 % with early detection, most patients are diagnosed at a late stage, leading to poorer prognosis and resulting in complications, such as the malfunction of mastication, deglutition, speech, and death [2].

The various treatment options for tongue carcinoma include surgery, chemotherapy, radiotherapy, and combined modalities. The treatment of choice depends on tumour factors, such as site, size (T stage), multiplicity, histological grade, and depth of invasion. Patient factors include the status of cervical lymph nodes, previous therapy, and medical condition of the patient [3,4]. Chemotherapy may potentially increase both survival and quality of life, improve prognosis, reduce distant metastasis, and inhibit tumour growth in this group of patients. One of the major factors in cancer treatment failure is the efficacy of current standard chemotherapy, such as

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5-fluorouracil (5-FU) and cisplatin (CIS), which is restricted partly due to their toxic side effects [5,6].

Natural substances derived from plants, known as phytochemicals, have been demonstrated to have a variety of positive effects on human health [7,8]. Recent research has emphasized the potential of phytochemicals as sources for cancer-related treatments. Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is a component of aloe vera and is a naturally occurring anthraquinone compound with an activity similar to that of anthracyclines. It shares this skeleton with mitoxantrone and daunorubicin, two antitumor antibiotics that can intercalate the DNA of cancer cells [9,10].

In addition to the well-documented antibacterial, anti-inflammatory, antiviral, and anti-ulcerogenic actions of emodin, some studies have clarified its anticancer effect. By blocking processes that result in treatment resistance, emodin may make tumour cells more susceptible to chemotherapy and radiation treatment. Emodin has also been shown to guard against toxicities linked to therapy [10,11]. Additionally, it has been shown that emodin blocks matrix metalloproteinase (MMP)-9 gene expression, which prevents the migration and invasion of human tongue cancer SCC-4 cells [12].

Few studies have examined the anticarcinogenic effects of emodin in oral human tongue cancer squamous cell carcinoma cell line (SCC25) cells. Thus, to benefit from reducing the concentration of the chemical anticancer medicine and eliminate any negative side effects on the body, this study focused on understanding this role and studying the advantages of using the natural product in treatment or combination with the usual chemotherapy.

2. Patient and methods

Research Ethics Committee approval for the Faculty of Dental Medicine for Girls Al-Azhar University was obtained (REC-CL-23-11).

The oral tongue SCC25 and normal lung epithelial cell line (WI38) were provided by the Holding Company for Biological Products and Vaccines (VACSERA, Egypt). The cells were imported from the 'American Type Culture Collection (ATCC)' in the form of a frozen vial. Emodin was purchased from Sigma Aldrich in Munich (Germany) in powder form. 5-Fluorouracil (5-FU) was provided by (VACSERA) in Egypt. Dulbecco's modified Eagle's medium (DMEM), 10 % foetal bovine serum (Hyclone), 10 ug/ml of insulin, 1 % penicillin-streptomycin, 0.25 % Trypsin EDTA and 100 µl complete growth medium. All the reagents were obtained from Sigma–Aldrich. Reagents of the in vitro viability assay kit included 3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) 15 mg/ml serum vial and MTT Solubilization Solution 10 % Triton X-100 plus 0.1 NHCl in anhydrous isopropanol; 125 ml was used for cytotoxicity testing.

Emodin was dissolved in methanol to a concentration of 5 mg/ml. SCC25 and WI38 cell lines were cultured in the VACSERA tissue culture laboratory. Cairo, Egypt. All the steps were performed in a laminar flow hood under aseptic conditions at 37 °C and pH 7.4. To dissociate the cells, the SCC25 and WI38 cell lines (stored in cryotubes) were removed from the liquid nitrogen container and promptly thawed in a water bath at 37 °C. Cells were cultivated in T25 flasks with 10 % heat-inactivated foetal bovine serum, 2 % sodium bicarbonate, and 2 % streptomycin penicillin. The cells were then incubated at 37 °C and 5 % CO_2 under incubator conditions. An inverted phase-contrast microscope was used to check the health of the cells regularly. Every two days, the medium was changed, and the culture was fed.

A popular colorimetric test for determining cellular metabolic activity is the MTT assay (Fig. 1). This widely used cell-based test can be used to assess cell proliferation, cytotoxicity, and activation in various cell types. It is quick and quantitative. The MTT assay relies on metabolically active cells that convert the yellow tetrazolium salt (MTT) into purple formazan crystals. Viable cells have NADH-dependent oxidoreductase enzymes that do this. The insoluble crystals were broken down using a solubilization solution, absorbance was assessed using an ELISA plate reader, and the more metabolically active cells present, the darker the colour of the resulting solution.

For the assay, cells were passaged at 5 × 103 cells per well and grown to greater than 80 % confluence before adding treatment as indicated in the experimental groups. Viable cells were determined 48 h later using the MTT assay. MTT has been validated

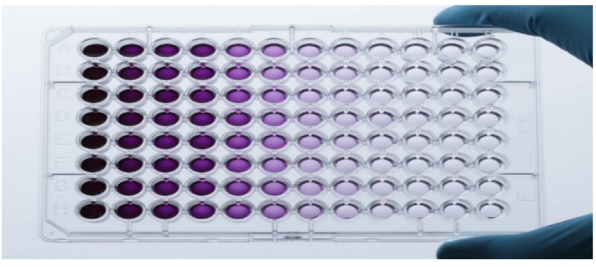

Fig. 1. A 96 well plate after 3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay. Decreasing the number of viable and metabolically active cells results in a decrease in the intensity of the purple color observed.

as an accurate measure of the viable cell population in cell culture, as well as the cytotoxicity of a tested compound added to cultured cells.

2.1. Statistical analysis

Data are shown as the mean ± standard deviation (SD). Data were examined for normality using the Kolmogorov–Smirnov test. The results of the Kolmogorov–Smirnov test showed that most of the data were normally distributed (parametric data); hence, an ANOVA test was employed to compare the groups. The independent *t*-test and Tukey's post hoc test for pairwise group comparisons were performed. The significance level was set at *P* less than 0.05. SPSS 19.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows was used to conduct statistical analysis.

2.2. Study design

The WI38 cell line was cultured for groups I and II, and emodin and 5-FU were applied at different concentrations to determine their IC50 values. The MTT cytotoxicity assay was used to determine the cytotoxicity of both substances on normal epithelial cells (WI38).

Groups III to VI: SCC25 cells were cultured, and emodin, 5-FU, and combinations of the two compounds with different ratios were applied at different concentrations to measure their IC50. The MTT cytotoxicity assay was performed to detect the cytotoxic effects of the materials on SCC25 cells.

3. Result

At 48 h, ANOVA revealed a highly statistically significant difference between groups ($P < 0.001$). Tukey's post hoc test showed that there was a statistically significant difference between groups, except for groups II and III, which were insignificantly different. For WI38, the IC50 of group I emodin/WI38 (106 ± 4.93) was higher than group II 5-FU/WI38 (49.0 ± 2.29) which means that lower concentration of 5-FU is more cytotoxic to normal cells than emodin. For SCC25, the highest IC50 mean values were in group III emodin (47.0 ± 2.28), then group IV 5-FU (12.50 ± 0.6), followed by group VI Emodin + low 5-FU (5.41 ± 0.26), with the least value was in group V emodin + high 5-FU (3.29 ± 0.16) which means that addition of emodin to either high or low concentrations of 5FU will decrease the concentration than if 5-FU or emodin used alone. They require lower concentrations to kill 50 % of the SCC25 cells than 5-FU or emodin alone (Table 1, Fig. 2).

Regarding cytotoxicity effects on WI38, emodin is less cytotoxic on normal epithelial cells by 54 % than 5-FU. For SCC25, the effectiveness of cytotoxicity of emodin + high 5-FU is increased by 74 %, while for

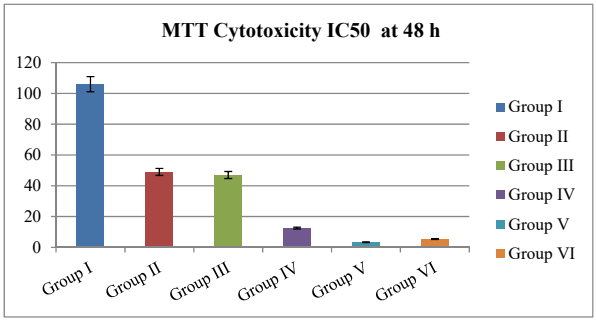

Fig. 2. Bar chart comparing the mean cytotoxicity of different groups at 48 h. MTT, 3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide.

Table 1. Descriptive statistics and comparison of all groups regarding IC50 values of cell cytotoxicity at 48 h by 3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay.

Groups	MW g/mol	WI38	
		Cytotoxicity IC50 uM at 48 h	
		Mean ± SD	Efficacy % by emodin
Group I: Emodin/WI38	270.34	106 ± 4.93A	
Group II: 5-FU/WI38	130.078	49.0 ± 2.29B	−54
		SCC25	
		Cytotoxicity IC50 uM at 48 h	
		Mean ± SD	Efficacy % by 5-FU[a]
Group III: Emodin	270.34	47.0 ± 2.28B	
Group IV: 5-FU	130.078	12.50 ± 0.6C	
Group V: Emodin + high 5-FU	2:1	3.29 ± 0.16E	74
Group VI: Emodin + low 5-FU	10:1	5.41 ± 0.26D	57
ANOVA test		791.506	
P value		<0.001	

Tukey's post hoc test; means sharing the same superscript letter are not significantly different.
[a] Value of efficacy at 48 h.

emodin + low 5-FU cytotoxicity is increased by 57 % more than 5-FU alone.

At 72 h, ANOVA revealed a highly statistically significant difference between groups ($P < 0.001$). Tukey's post hoc test showed that there was a statistically significant difference between the groups. For WI38, the IC50 of group I [Emodin/WI38 (93.4 ± 3.56)] is higher than group II [5-FU/WI38 (45.8 ± 1.48)] which means that lower concentration of 5-FU is more cytotoxic to normal cells than emodin. For SCC25, the highest IC50 mean values were in group III [Emodin (38.6 ± 3.56)], then group IV [5-FU (7.62 ± 0.29)], followed by group VI [Emodin + low 5-FU (6.91 ± 0.26)], with the least value was in group V [Emodin + high 5-FU (2.89 ± 0.11)] which means that addition of emodin to either high or low concentrations of 5-FU will decrease the concentration than if 5-FU or emodin were used alone. They require fewer concentrations to kill 50 % of the SCC25 cells than 5-FU or emodin alone (Table 2, Fig. 3).

Regarding cytotoxicity effectiveness on WI38 (WI-38 is a diploid human cell line composed of fibroblasts derived from lung tissue of a 3-month-gestation female fetus), emodin is less cytotoxic on normal epithelial cells by 51 % than 5-FU.On SCC25, the effectiveness of cytotoxicity of emodin + high 5-FU is increased by 62 %, and emodin + low 5-FU cytotoxicity was increased by 9 % compared with 5-FU alone.

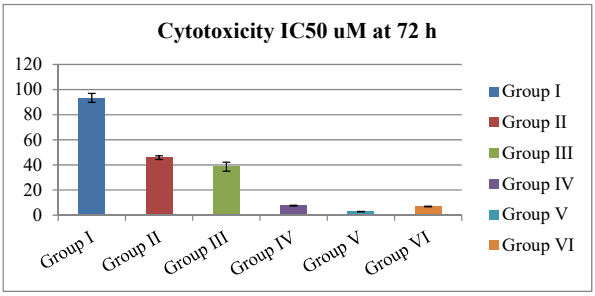

Fig. 3. Bar chart illustrating the mean cytotoxicity at 72 h in different groups.

4. Discussion

Malignant tumours seriously threaten people's health and life worldwide. Oral cancer ranks eighth among the most common causes of cancer-related deaths in the world. Oral SCC (OSCC) is the most common type, accounting for over 90 % of oral cancers, with the tongue being the most commonly affected site [1]. Chemotherapy is one of the most popular treatment methods that can be considered the first option, particularly in patients who cannot tolerate surgical treatment. One of the most often prescribed chemotherapy drugs, 5-FU, remains a crucial treatment for several solid tumours, including OSCC. However, the systemic use of 5-FU during tumour therapy causes serious toxic side effects and the emergence of drug resistance has thus restricted the function of 5-FU [13].

Therefore, in this work, we introduced a method that would involve emodin being added to 5-FU to lower the chemotherapeutic concentration while maintaining therapeutic efficacy.

Emodin was selected as the focus of this study because it has been investigated in several sectors, including the treatment of cancer, and because it has demonstrated promising results with several cancer types. Emodin suppresses prostate cancer cell proliferation by downregulating androgen receptors [14] and by blocking the coalescence of lipid rafts, interfering with integrin clustering, and

Table 2. Descriptive statistics and comparison of all groups regarding IC50 values of cell cytotoxicity at 72 h by 3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide assay.

Groups	MW	WI38	
		Cytotoxicity IC50 uM at 72 h	
		Mean ± SD	Efficacy % by emodin
I: Emodin/WI38	270.34	93.4 ± 3.56A	
II: 5-FU/WI38	130.078	45.8 ± 1.48B	−51 %
		SCC25	
		Cytotoxicity IC50 uM at 72 h	
		Mean ± SD	Efficacy % by 5-FU[a]
III: Emodin	270.34	38.6 ± 3.56C	
IV: 5FU	130.078	7.62 ± 0.29D	
V: Emodin + high 5-FU	2:1	2.89 ± 0.11F	62
VI: Emodin + low 5-FU	10:1	6.91 ± 0.26E	9
ANOVA test		789.298	
P value		<0.001	

Tukey's post hoc test: means sharing the same superscript letter are not significantly different.
[a] Value of efficacy at 72 h.

preventing the formation of focal adhesion complexes. It also prevents the adherence of human breast cancer (MDA-MB-231) [15], human cervical epithelioid carcinoma (HeLa), and human hepatocarcinoma (HepG2) tumour cells [16].

To judge the beneficial action of emodin on SCC25, it was essential to use a reference chemotherapeutic drug for comparison of emodin results with its results. 5-FU is regarded as one of the most powerful antineoplastic agents against several common malignancies, including colon, breast, skin, and oral cancers [10], and it was chosen as a reference in the current study.

The MTT cytotoxicity test was used to determine the concentration of compounds with strong cytotoxic effects based on their IC50 (IC50 is Half-maximal inhibitory concentration) values. This assay is frequently employed to assess medication efficacy and potency in cells exposed to a drug for up to 72 h. It is simple to use and safe [17,18].

Considering the IC50 results for SCC25 in this study, there was a highly statistically significant difference between the groups ($P < 0.001$). The statistically highest mean IC50 value was with group III (emodin) (47.0 ± 2.28 and 38.6 ± 3.56 uM), followed by group IV (5-FU) (12.50 ± 0.6 and 7.62 ± 0.29 uM), then in group VI (emodin added to low 5-FU concentration) (5.41 ± 0.26 and 6.91 ± 0.26 uM) while the least value was in group V (emodin added to high 5-FU concentration) (3.29 ± 0.16 and 2.89 ± 0.11 uM) at 48 and 72 h, respectively, which means that addition of emodin to either high or low concentrations of 5-FU will decrease the concentration than if 5-FU or emodin used alone.

These findings are consistent with those of Tian et al. [19], who used the MTT assay to show that emodin has anticancer activity against MKN45 (MKN-45 cells are a human gastric adenocarcinoma cell line) gastric carcinoma cells in a time- and concentration-dependent manner. They discovered that 5-FU and emodin together significantly increased the growth inhibitory effect compared with 5-FU and emodin alone. For instance, when 20 μg/ml emodin was mixed with 10 mg/l 5-FU and applied to MKN45 cells for 72 h, the inhibition rate was twice as high as that of the 20 μg/ml emodin group and more than twice as high as that of the 5-FU group. This showed that emodin has an antitumour effect in addition to having a cytopathic effect on the gastric cancer cells MKN45.

Additionally, Lui et al. [20] demonstrated a synergistic impact of low concentration emodin and 5-FU treatment given one after the other, which caused cellular senescence in breast cancer (MCF-7 (MCF-7 is a human breast cancer cell line)) cells. Low-concentration emodin-enhancing breast cancer chemotherapy requires the absence of notch-regulated ankyrin repeat protein (NRARP), indicating that the potential clinical benefits of incorporating emodin into standard chemotherapy regimens warrant consideration in the treatment of refractory and relapsed breast cancer patients.

These findings are consistent with previous research showing that emodin has cytotoxic and growth-inhibitory effects on various tumour cell types. It exhibits these effects on several cell lines using various ways [21]. There is growing clinical evidence suggesting that inflammation contributes to the development of cancer, and that the link between inflammation and genes is critical early on. Since inflammation almost always results in tumours, it is well recognized that if inflammatory mediators result in cancer, treating the inflammation may be the first step in treating cancer. Since emodin has anti-inflammatory properties, its anticancer effects have also been examined [22,23].

Emodin could also make HL-60/ADR cells more sensitive to multiple drug resistance [24]. When combined with Azidothymidine, Emodin had an inhibitory effect on the proliferation of the K562/ADM cells [25], Additionally, emodin is capable of treating 5-FU-resistant breast cancer [26,27].

To explore the material that shows the lower cytotoxic effect on normal epithelial cells according to their IC50 values in the present study, emodin and 5-FU were applied on WI38 for the same incubation periods, and the results showed that the IC50 of group I emodin/WI38 (106 ± 4.93 and 93.4 ± 3.56 uM) is higher than group II 5-FU/WI38 (49.0 ± 2.29 and 45.8 ± 1.48 uM) at 48 and 72 h, respectively, which means that lower concentration of 5-FU is more cytotoxic to normal cells than emodin. Similarly, Cotoraci C [28], using MTT, discovered that emodin had very significant cytotoxicity against the chronic myelocytic leukemia (K562) cell line, but not against normal human embryonic kidney (293) cells, indicating that it had tumour-selective growth inhibitory effects.

4.1. Conclusion

From the previous results, the following conclusions could be drawn: emodin exerts apoptotic effects on the tongue SCC cell line similar to 5-FU in a time-dependent manner, the combination of emodin with 5-FU can potentiate its action in the treatment of OSCC, and emodin can be used as co-treatment with chemotherapy in the treatment of OSCC to avoid its negative effects.

4.2. Recommendations

(a) To employ emodin as a chemotherapeutic agent in the future, further research needs to be conducted to test it against more cancer cell lines.
(b) Additional research should be conducted to use low concentrations of 5-FU to prevent the hazardous side effects of 5-FU.

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Ethics information

The Ethics Committee of the Faculty of Dental Medicine for Girls Al-Azhar University accepted the research, which was carried out at the confirmatory diagnostic facility at VACSERA in Egypt.

Biographical information

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Conflict of interest

There was no conflict of interest.

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