RESEARCH ARTICLE

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Anti-Cancer Effects of Traditional Medicinal Herbs on Oral Squamous Cell Carcinoma

Ali Dehghani Nazhvani[1,2], Najmeh Sarafraz[3], Fatemeh Askari[4], Fahimeh Heidari[5], Mahboobeh Razmkhah[6]*

Abstract

Backgrounds: Oral squamous cell carcinoma (OSCC) is among the most frequent oral cancers in individuals under 40. Documents have endorsed that a diet enriched with fruit and vegetables can banish the risk of developing major cancers. This study aimed to evaluate the effects of different concentrations of four medicinal herbs including saffron, ginger, cinnamon and curcumin on OSCC cell line. **Methods:** Having obtained the aqueous extract of the four herbs, they were administered on OSCC cell lines per se and in dual, triple, and quadruple combinations. Their effects were measured in different concentrations and in 24 and 48 hours by using MTT assay. **Results:** The minimum and maximum effective concentrations were respectively 108 and 217 mg/ml for curcumin with IC30 of 77mg/ml, 108 and 270 mg/ml for ginger with IC30 of 58 mg/ml, 2 and 10 mg/ml for saffron with IC30 of 1.9 mg/ml, and 5 and 40 mg/ml for cinnamon with IC30 of 3.3 mg/ml. The best effect of the combinations was seen for cinnamon-saffron after both 24 and 48 hours and the four herbs combination after 48 hours. **Conclusion:** Although all the four herbs were effective on OSCC cell line, the strongest extract was saffron, followed by cinnamon. Combination of cinnamon-saffron and combination of the four herbs showed maximum effects. These findings suggest that traditional medicinal herbs may potentially contribute to oral cancer treatment; providing new windows for the development of new therapeutic strategies for OSCC.

Keywords: Oral squamous cell carcinoma- Saffron- Cinnamon- Curcumin- Ginger

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Introduction

Oral squamous cell carcinoma (OSCC) is among the most prevalent oral cancers, the risk of which is increasing in individuals under 40 years old, particularly in developed countries (Aghbali et al., 2013). The proposed risk factors include micronutrient deficiencies, chronic trauma, poor oral hygiene, and some specific microorganisms like viruses (Lauritano et al., 2016). Although recurrence is common, the chief treatment of OSCC is through surgery and radiotherapy (Aghbali et al., 2013).

Herbal medicines are plant-based products used as the traditional and domestic medicines and food additives. Currently, the pharmaceutical properties of these herbs are being vastly investigated. It is proposed that the medicinal herbs have the potential to treat several diseases such as cancer, allergy, and diabetes (Kwon et al., 2010). Evidence has endorsed that people with a diet rich in fruits and vegetables are less likely to develop major cancers (Buch et al., 2012). Hence, the need for new medicines has led to seeking anti-cancer effects in fruits and vegetables (Gutheil et al., 2012).

Curcumin is a phytopolyphenolic pigment derived from turmeric curcuma longa which is usually added to food as flavor (Kim et al., 2012). Buch et al., (2012) reported that this plant induced the phagocytosis of free radicals and inhibited lipid peroxidation.It is believed that curcumin decreases the proliferation of tumoral cells. It suppresses the tumor angiogenesis, and consequently reduces the tumor growth and metastasis. Goel et al., (2001) reported that curcumin prevented the COX2 production in human colon cancer cells. Kuttan et al. , (1987) observed that the ethanol extract of turmeric like topical curcumin completely healed the cancer symptoms.

Ginger (Zingiberofficinale Roscoe) is a popular spice particularly in Asia and contains abundant bioactive components that promote the health (Ghasemzadeh et al., 2010). Ginger oil scavenges superoxide and hydroxyl radicals, and inhibits lipid peroxidation in vitro. It considerably decreases the dextran- and carrageenan-induced acute inflammation (Jeena et al., 2013). These features make the ginger an effective agent

*[1]Department of Oral and Maxillofacial Pathology, [2]Biomaterials Research Center, [3]Department of Periodontics, School of Dentistry, [4]Department of Phytopharmaceuticals, School of Pharmacy, [5]Department of Molecular Medicine, School of Advanced Medical Sciences and Technologies, [6]Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. *For Correspondence: razmkhahm@sums.ac.ir*

in preventing UV-B-induced production of reactive oxygen species (ROS) and COX2. Hence, it can be used as a therapeutic agent in UV-induced skin diseases (Ali et al., 2008).

Saffron is naturally taken from the stigma of the crocus sativus flower (Manoharan et al., 2013). It has been reported that saffron is an antioxidant, anti-cancer, anti-inflammatory, antidepressant, antihistamine, and memory enhancer (Harrington, 2011) and its anti-cancer effects have been confirmed in vitro and in animal models (Zhang et al., 2013).

Cinnamon is the outermost bark of an evergreen tree belonging to the Lauraceae family. Its extract contains several active components such as essential oils and tannin. They have numerous biological functions such as antioxidant, antibacterial, anti-inflammation, anti-diabetes, and anti-tumor activity. Oral consumption of cinnamon extract in animal melanoma transplantation model considerably inhibited tumor growth (Kwon et al., 2010).

Aromatic plants like cinnamon also contain eugenol which is considered in the allylbenzene class of chemical compounds. Recent studies have confirmed the anti-cancer effect of eugenol against various cancer cell lines in various animal models. Moreover, the molecular mechanism of eugenol-induced apoptosis is well documented in melanoma, skin tumors, osteosarcoma, leukemia, gastric cancer and mast cells (Jaganathan and Supriyanto, 2012). As previously mentioned, these medicinal herbs have significant anti-cancer properties but few studies was found on OSCC. Hence, the present study was performed to quantitatively assess the anti-cancer effect of curcumin, ginger, saffron, and cinnamon per se and in combination on a cell line of oral squamous cell carcinoma.

Materials and Methods

In this study, ginger, curcumin, cinnamon, as well as saffron were administered to OSCC cell line named KB obtained from Pasteur Institute of Iran.

Taking the aqueous extract

The herbs, ginger, curcumin, cinnamon, and saffron, were ground, weighed, and soaked in distilled water (1:20) for 48 hours. Then, the mixture was passed through cleansing napkin so that the waste would be separated. The obtained liquid from each plant was passed through filter paper by using Büchner funnel and vacuum pump. The solutions were poured in separate flasks of rotary devices. After nearly 3-4 hours, condensed extracts were obtained and centrifuged at 10,000 rpm. After 15 minutes, the supernatant was cautiously separated by sampler and passed through 0.22-µm filter to eliminate the microorganisms and achieve sterile extracts. The extracts were then aliquoted, poured in 10 mL falcons, and stored at -80°C.

Thiazolyl Blue Tetrazolium Bromide (MTT) assay

KB cells were cultured in RPMI (Roswell Park Memorial Institute 1640) medium. After the third passage, 10,000 OSCC cells were trypsinized and transferred to the wells of a 96-well plate. They were allowed to adhere to the bottom of the plate and be stabilized.The cells with a spectrum of concentrations of the four herbs were incubated to measure their effects after 24 and 48 hours and then IC30 was evaluated using MTT assay. The control wells were cells cultured in standard condition without any treatment.

Briefly, the culture media were completely removed and 200µl 0.1% MTT solution in complete culture medium were added.Cells were incubated for 4hrs at 37°C in 5% humidified CO_2 incubator. Next, 150 µl of MTT solvent (DMSO) were added to each complete dried well. Ultimately, the absorbance was determined with spectrophotometer at 570 nm wavelength. Control and test groups were statistically compared by using Kruskal-Wallis and Dunn's multiple comparison tests. IC30 was calculated using CurveExpert Professional software.

Results

This study investigated the anti-cancer effects of four medicinal herbs including curcumin, saffron, ginger, and cinnamon on OSCC.

The findings showed that the effect of curcumin began from the concentration of 108 mg/ml after 24 hours and at the concentration of 217 mg/ml over 50% of the cells died. Similarly, the lowest effective concentration of curcumin after 48 hours was observed in 108 mg/ml and the highest was in 217 mg/ml (Figure1). The curcumin extract's IC30 at 48 hours post treatment was 77 mg/ml.

Regarding the 24-hour assessment of ginger, the anti-cancer effect initiated in 108 mg/ml. It reached 96% efficacy in 270 mg/ml. The 48-hour assessment showed that the effect began in 54 mg/ml and reached 100% in 270 mg/ml (Figure1). The IC30 of ginger extract at 48 hours post treatment was 58 mg/ml.

Assessment the impact of saffron on KB cells within 24 hours showed that it was effective in 2 mg/ml. It killed nearly 50% of cells in 5 mg/ml concentration. The maximum effect (100%) was observed in 10 mg/ml.In 48-hour assessment, the effective concentration began in 1 mg/ml, whereas IC50 was observed in 5 mg/ml. The maximum impact (100%) of saffron was achieved in 10 mg/ml and the saffron IC30 after 48 hours was 1.9 mg/ml (Figure1).

In 24-hour assessment of cinnamon, the anti-cancer effect was first seen in 5 mg/ml, and 80% effectiveness was seen in 10 mg/ml. Ultimate effect (100%) was observed in 40 mg/ml after 24 hours. Using cinnamon in the OSCC cells culture medium for 48 hours showed that cinnamon began to be effective in 5 mg/ml and the cinnamon IC30 after 48 hours was 3.3 mg/ml (Figure 1).

Moreover, 24-hour and 48-hour evaluations of the IC30 of dual, triple, and quadruple combination of these herbs showed that the highest anti-cancer effect was seen in cinnamon-saffron combination, and the lowest effect was seen in cinnamon-ginger combination (Figure 2).

Statistical analyses revealed significant differences ($P<0.05$) among the 24-hour effects of cinnamon-saffron,

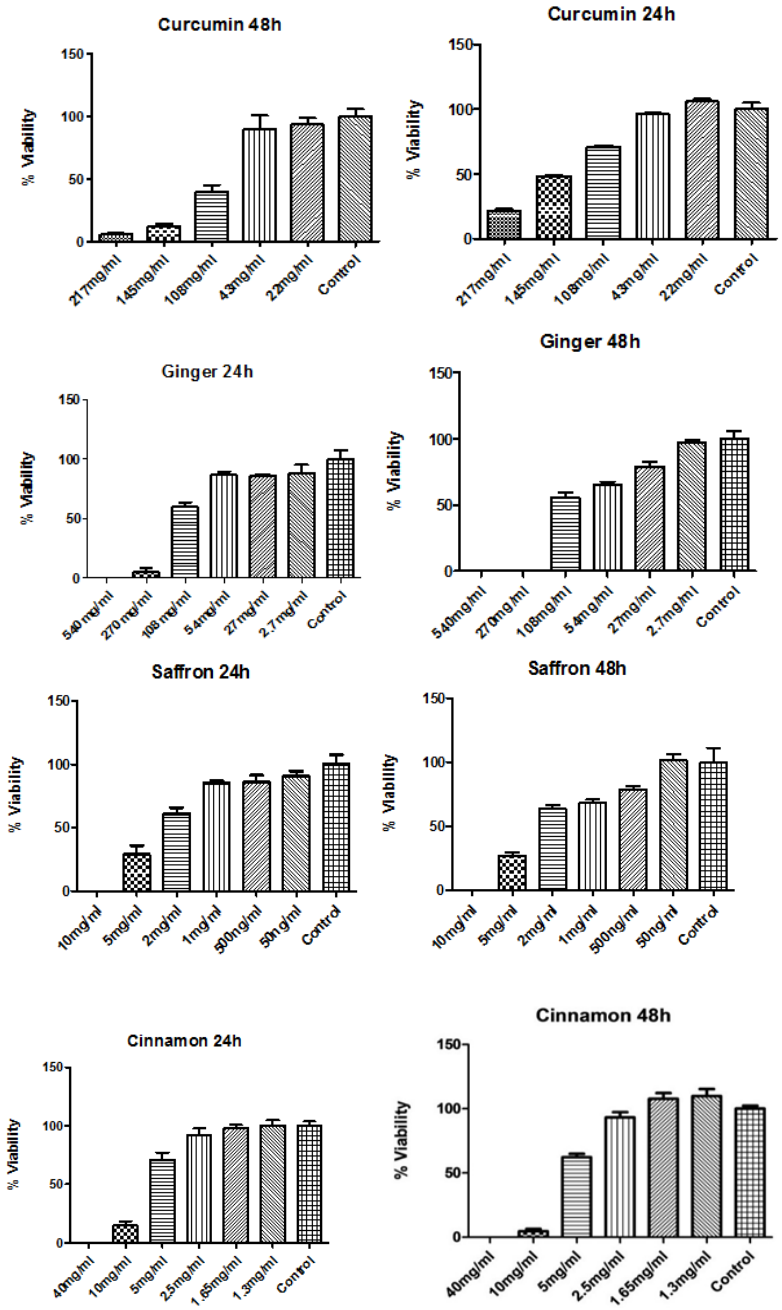

Figure 1. Effective Concentration (mg/ml) of Curcumin, Saffron, Ginger, and Cinnamon on OSCC Cell Line after 24 and 48 hrs.

cinnamon-curcumin-saffron, saffron-curcumin-ginger, and the quadruple combination of the four herbs versus the control group, and also cinnamon-ginger combination versus cinnamon-saffron, curcumin-cinnamon-saffron, and the quadruple combination of the four herbs (Figure 2).

The findings also revealed significant differences ($P<0.05$)between the 48-hour effects of the cinnamon-saffron, cinnamon-saffron-ginger, cinnamon-saffron-curcumin, and the quadruple combination of the herbs versus the control group. Likewise, significant differences were observed between the combination of cinnamon-saffron versus the combination of cinnamon-ginger, saffron-ginger, cinnamon-ginger-curcumin, and also between cinnamon-ginger, cinnamon-ginger-curcumin versus the combination of all the four herbs (Figure 2).

Discussion

Oral squamous cell carcinoma has become a major global concern. Its standard treatment method highly depends on the clinical stage of the disease. In early stages, the disease can be primarily treated through surgery or radiotherapy; whereas the advanced stages might require combination of surgery, radiotherapy and/or chemotherapy (Aghbali et al., 2013). OSCC treatments are generally associated with several side effects. Surgery causes loss of functionality while radiotherapy usually causes mucositis, oral candidiasis, loss of gustatory sensation, and xerostomia (Vissink et al., 2003). Also, radiotherapy-induced osteoradionecrosis generally occurs due to the damages to intraosseous vessels in the radiation area

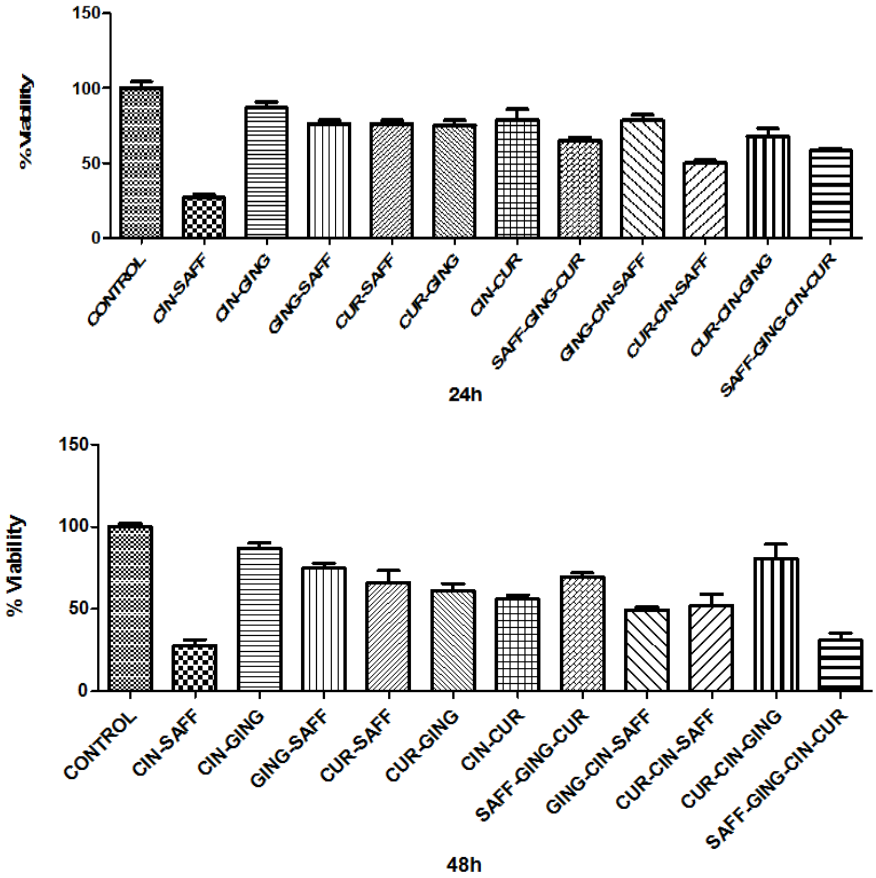

Figure 2.Comparison of Dual, Triple, and Quadruple Combination of Studied Herbs by Their IC30s in 24 and 48-hour Evaluation.

(Thorn et al., 2000). Chemotherapy drugs have many draw backs on normal cells leading to systemic and complications, as well as resistance to OSCC treatment. The role of medicinal herbs as anti-cancer agents has been recently investigated by several researchers (Buch et al., 2012; Dhillon et al., 2008; Koppikar et al., 2010). The present study focused on the anti-cancer effect of cinnamon, curcumin, saffron, and ginger against the OSCC cell line and tried to compare the effective concentration of these herbs with each other.

Curcumin is a perennial herbaceous plant that grows up to 1-1.5 m in height, with swollen rhizome out of which grows a number of aerial stems. It has several proven effects such as anti-inflammatory, and inhibition of mucosal injury (Bimonte et al., 2016). Bimonte et al., (2016) mentioned the satisfactory therapeutic effects of curcumin against pancreas cancer. Kim et al., (2012) concluded that curcumin administered its anti-cancer effects against OSCC through autophagy and apoptosis mechanisms. In the present study, although curcumin showed cytotoxic/preventive effects on OSCC cell line, its effect was weakest compared to the other herbal extracts studied.

Ginger is one of the oldest oriental herbs and spices whose rhizome has always been regarded as medicine with outstanding properties. It has antiemetic, anti-inflammatory, anti-oxidation, and anti-clotting effects, among lots of other benefits (Ali et al., 2008; Ghasemzadeh et al., 2010). Having thoroughly investigated this herb, Suresh et al., (2010) described the anti-tumor mechanism of action of ginger through anti-oxidation and anti-lipid peroxidation, besides improving the level of detoxifier enzymes. Lionel et al., (2012) studied the inhibitory effect of a derivation of Malaysian ginger, 1'-Acetoxychavicol acetate, on the growth of OSCC through inducing apoptosis, inhibiting the activation of NFκB and decreasing the COX2 and Cyclin D1 expression. Consistently, we reported the anti-cancer effect of ginger on OSCC cell line as it was ranked third after saffron and cinnamon.

Saffron is a perennial herbaceous plant belonging to the Lily Family within the Lillian's order. Manoharan et al., (2013) described the anti-tumor mechanism of saffron through restoring the detoxifier enzymes, lipid peroxidation suppliers, and antioxidants to the normal level. Tavakkol Afshari et al., (2008) reported IC50 of 800 µg/ml against HeLa and 950 µg/ml against HepG2 cell lines for saffron within 48 hours. In the current study the effective concentration of saffron was lower than the other medicinal herbs studied, thus saffron may have the highest anti-cancer effect for OSCC.

Cinnamon extract contains several active components such as essential oils and tannin with many biological functions like antioxidation, antimicrobial, anti-inflammatory, anti-diabetes, and anti-tumor activity (Kwon et al., 2010). Kwon et al., (2010) study came to the conclusion that the anti-cancer effect of cinnamon extract was directly related to the

augmentation of apoptosis and inhibition of NFκB and AP1 activity. Yang et al., (2015) found that the cinnamon essential oil acted as a chief anti-cancer agent against head and neck squamous cell carcinoma. Herdwiani (2016) reported that cinnamon acts as an anti-cancer agent against various types of cancer cells such as basal cell carcinoma, breast cancer cell line (MCF7), epidermoid carcinoma (A431), and human OSCC (Ca9-22 and SCC12). The present study showed that cinnamon had the highest anti-cancer effect after saffron; the minimum effective concentration of cinnamon on OSCC was 5 mg/ml in 24 and 48 hours assessment. It was also found that this extract reached 90% cytotoxicity/prevention in 10 mg/ml within 48 hours.

Evaluating the effect of dual, triple, and quadruple combination of these herbs yielded interesting findings regarding the anti-cancer effect of the combination of these extracts compared with the single use. The 24-hour evaluation of these combinations revealed the highest effect in cinnamon-saffron combination; which was even more effective than the combination of the four extracts. Similarly, in 48-hour evaluation, the highest effect was observed in cinnamon-saffron combination, followed by combination of all the four extracts. The increased effect of the quadruple combination compared with its counterpart in 24-hour evaluation indicated that the combination of all the four herbs can be more effective as the time passes, just like cinnamon-saffron combination.

The most important limitation of our study was not to investigate the type of effects of medicinal herbs studied because the results of MTT assay can reflect the number of viable cells present or evaluate cytostatic activity (shift from proliferation to quiescence). Thus, further experiments such as proliferation and cell cycle analysis, apoptosis assessment and detection of autophagy are recommended for better elucidation.

In conclusion, it can be concluded that all the studied herbs (ginger, curcumin, saffron, and cinnamon) had anti-cancer effects on OSCC cell line among which saffron had the strongest effect compared to the other studied herbs. Moreover, the combination of saffron-cinnamon and cinnamon-saffron-curcumin-ginger had the highest effects compared to the combinations studied. This study had better extend in future to assess the anti-tumor molecular mechanism of action of traditional medicinal herbs in OSCC. The findings of this study strengthen the hopes for establishing novel low-cost therapeutic strategies based on the use of medicinal herbs.

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Conflict of interest

The authors declare no conflict of interest.

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