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Research article

Baicalein inhibits KB oral cancer cells by inducing apoptosis via modulation of ROS

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ABSTRACT

The theme of the current examination was to evaluate the anticancer activity of baicalein (5,6,7-trihydroxyflavone) in human oral cancer KB-cells by observing cell restraint intercellular ROS movement and apoptotic changes using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) test. The concentration of baicalein at 80 µM caused 100% cancer cell inhibition. Therefore, lower concentrations i.e. 10, 20 and 40 µM were selected in the present study. Baicalein at 40 µM significantly hindered human oral cancer KB cells. In the interim, incendiary and apoptosis were seen through expanded ROS action that actuated apoptotic cell death. The results of the present study suggested that the anticancer effect of baicalein is likely due to its potential to improve ROS level and hence the increased apoptotic activity. This study suggested that baicalein might be a potential candidate for the treatment of oral cancer.

INTRODUCTION

Oral cancer, one of the most widely recognized harmful neoplasms around the world, begins as an uncontrolled development of cells in the mouth and prompts disfigurement of the face, debility of body and in the long run demise. It is the fifth most incessant cancer worldwide and influences above 500,000 new cases every year (Mangalath et al., 2014). A larger part of oral cancers (90%) emerge in the squamous epithelium of the oral pit and are normally gone before (Choi and Myers, 2008). While oral squamous cell carcinoma is the most widely recognized worldwide cancer, this type of cancer containing roughly 33% of all cancers in Central and South-East Asian nations. Epidemiological, test and clinical investigations brought up that tobacco smoking, smokeless tobacco items, alcohol utilization, HPV contaminations, betel quid biting and low products of the soil admission are the hazard elements of oral cancer (Ram et al., 2011). Oral cancer patients, because of the asymptomatic nature of oral cancer at introductory stages, commonly look for clinical consideration when the malignancy is at a propelled stage. The endurance pace of oral cancer patients is likewise because generally analysis, in spite of late progression in oral cancer treatment. Despite broad examination, the clinical result and anticipation of OSCC are as yet not great; over half of patients bite the dust of this illness or complexities in 5 yrs (Geum et al., 2013). It has been accounted for that oral cancer is answerable for most elevated mortality proportions among all malignancies. In this manner, scanning for new remedial techniques has been the most basic and rising issue. While looking through restorative specialists fit for impeding the cell cycle in OSCC.

Chemoprevention is a valuable way to deal with discover the counter tumour starting or hostile to tumour advancing capability of characteristic items and engineered operators. Chemopreventive operators smother, hinder, stop or converse the procedure of carcinogenesis either by repressing the metabolic actuation of cancer-causing agents or by upgrading the detoxification of extreme cancer-causing metabolites. Additionally, they stifle tumour development by hindering the unreasonable age of ROS and by improving the cancer prevention agent barrier system.

Flavonoids are the regular substance generally conveyed in grains, natural products, vegetables, leaves, barks, stems, roots and blossoms. With more than 4000 variable phenolic structures, flavonoids have been found from plant species

(Middleton, 1998). It has been shown through various logical examinations that the flavonoids have antibacterial, hostile to angiogenesis, calming, antiviral, antiatherosclerotic, antitumour, against thrombogenic, and antifungal movement (Panche et al., 2016). Numerous investigations have likewise detailed the astounding and critical properties of flavonoids as anticancer and additionally chemopreventive specialists, recommending a positive relationship between a lower danger of malignant growth and a flavonoid-rich eating routine (Bondonno et al., 2019). Baicalein is a bioactive specialist and one of the flavonoid mixes which is essentially detached from the base of *Scutellaria baicalensis* Georgi also called Scute, a natural plant of the family Lamiaceae where the roots have therapeutic properties. Clinical investigations on baicalein have indicated that it has pharmacological exercises including antiviral action and photoprotective impacts against oxidative pressure. It is additionally appeared to mitigate fiery response, animate typical skin keratinization and lessen liver inflammation and fibrosis. Be that as it may, the anticancer activity of baicalein on oral disease stays indistinct. In this examination, we planned to explore the impact of baicalein on oral malignancy through a balance of ROS.

MATERIALS AND METHODS

Chemicals and reagents

Dulbecco's Modified Eagles Medium (DMEM), Phosphate Buffered Saline (PBS), fetal bovine serum (FBS), 0.25% trypsin EDTA, antibiotics (penicillin, streptomycin), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 2,7-diacetyl dichlorofluorescein DCFH-DA), Ethidium Bromide (EtBr), Rhodamine 123, Acridine Orange (AO), Hoechst 33342 stain were obtained from Hi-media Lab Ltd., Mumbai, India. Baicalein (Fig 1) was kindly gifted by Dr. Bakrudeen Ali Ahmed Abdul, Faculty of Applied Science, Ton Duc Thang University, Ho Chi Minh, Vietnam.

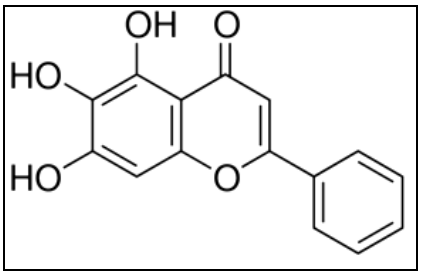

Fig. 1. Chemical structure of baicalein

Cell culture

The Human pharyngeal carcinoma (KB) cell line was purchased from NCCS, Pune, India. The KB cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) and maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air incubation. Baicalein freshly dissolved in 1% DMSO before the treatment.

Cell proliferation assay

The effect of baicalein on the cell proliferation of KB-cells was determined by MTT assay based on the detection of mitochondrial dehydrogenase activity in a healthy cell. KB-cells were seeded in 96-well plates at a density of $5x10^3$cells/well in a final volume of 100ml with DMEM and incubated up to 24 h. The cells were treated with different concentration of baicalein. After 24 h, the cells were incubated with 100 ml of MTT solution (1 mg/ ml) for 2 h at 37 °C. The MTT solution was removed and added 100 ml of DMSO to dissolve the formazan crystals. The plate was read at 570 nm in a Read well touch, ELISA plate reader (Robonic, India).

Measurement of intracellular ROS generation

Intracellular ROS was measured by using a non-fluorescent probe, DCFH-DA that can freely penetrate into the intracellular matrix of cells where it is oxidized by ROS to fluorescent dichlorofluorescein (DCF). Thus, the fluorescence intensity is directly proportional to the amount of ROS generation. The cells were seeded ($1x10^6$ cells/well) in 6-well plate treated with baicalein at different concentration and kept in a CO_2 incubator for 24 h. After 24 h of incubation, 1 ml of cells were incubated with 100 ml of DCFH-DA for 10 min at 37 °C. Fluorescent intensity was measured with excitation and emission filters set at 485 and 530 nm, respectively (Shimadzu RF-5301 PC spectrofluorometer). The results were articulated as the percentage increase in the fluorescence intensity.

Statistical analysis

Data are expressed as mean ± standard error (SE) for a minimum of three independent determinations in triplicate for every experimental point. Data were analyzed using SPSS Statistics software. For all the measurements, one-way analysis of variance followed by Duncan's new multiple range test ($p \leq 0.05$) was used to assess the statistically significance of the difference between control and treated groups.

RESULTS

Cytotoxic effect of baicalein

The results of the cytotoxic effect of baicalein on KB-cells are shown in Table 1. The cells were treated with different concentrations of baicalein (5-100 µM) for 24 h incubation, which revealed a

dose-dependent inhibition of cell proliferation. Maximum cell death was observed at 80 µM concentrations. Hence, the inhibitory concentration 50 (IC_{50}) of baicalein for KB-cells 40 µM apparent from growth inhibition curve, we selected 10, 20 and 40 µM doses of baicalein for further studies.

Table 1. Inhibitory effect of baicalein in KB-cells

Replication	Control	10 µM	20 µM	40 µM
1	39.8 ± 4.1	27.3 ± 2.4	32.7 ± 3.4	19.5 ± 1.5
2	39.2 ± 4.0	24.8 ± 2.0	31.8 ± 3.1	18.5 ± 1.3
3	39.0 ± 3.9	25.0 ± 2.2	32.0 ± 3.2	17.2 ± 1.0

Values are expressed as Mean ± SE.

Effect on intracellular ROS generation

The intracellular ROS generation was measured by DCFH-DA staining. The levels of ROS generation in control and baicalein treated cells are shown in Fig. 2. KB-cells were treated with different concentration of baicalein (10, 20 and 40 µM) shows significantly increased levels of ROS generation which indicating extreme green fluorescence intensity as compared to untreated control cells.

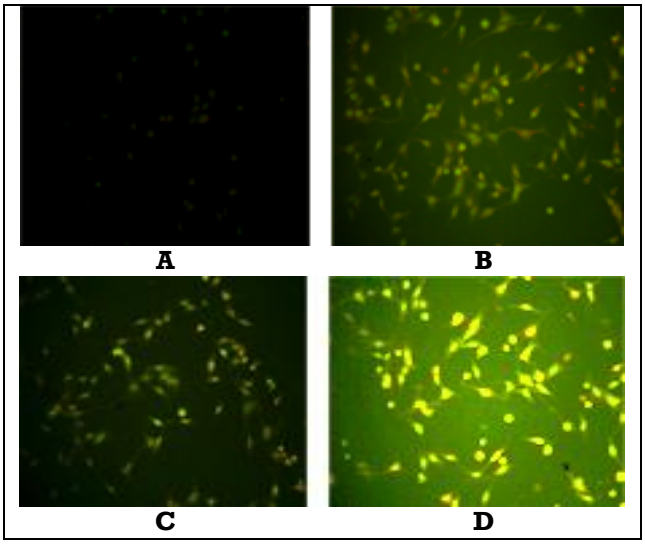

Fig 2. Effect of baicalein on intracellular ROS generation in KB-cells using DCFH-DA staining. Photo micrographic image of A: Control shows weak fluorescence; B: Treatment with 10 µM shows mild fluorescence; C: Treatment with 20 µM shows moderate fluorescence; D: Treatment with 40 µM shows enhanced fluorescence indicating increased ROS generation.

DISCUSSION

Phytochemicals are pulled in among established researchers for improvement of chemotherapeutic medication. Epidemiological examinations propose that standard utilization of naturally happening phytonutrients limit the occurrence of cancer movement. For instance, 60% of anticancer medications are created from the natural root (Lichota and Gwozdzinski, 2018). Past investigations have been accounted for the flavonoid mixes, for example, apigenin (Madunic et al., 2018), curcumin (Tomeh et al., 2019) just as coumarin (Kaur et al., 2015) displays anticancer impact against different cancer cell line. In this investigation, we inspected, baicalein represses cell proliferation and acceptance of apoptosis in KB cells. Our outcomes unmistakably demonstrated that baicalein adequately restrains cell proliferation at a focus depended way. Be that as it may, baicalein showed negligible poisonousness on ordinary gingival fibroblast cells at a convergence of upto 80 µM. It might fill in as a possibly significant contender for cancer chemotherapy. Past investigations demonstrated that baicalein represses cell proliferation, for example, ovarian, bosom, lung, colon, and skin cancers (Ninfali et al., 2017). These confirmations reliable with our outcomes additionally show essentially more powerful antiproliferative impacts of baicalein.

Oxidative pressure is an asymmetry between the age and additionally, disposal of receptive oxygen species, cause outrageous oxidative harm to macromolecules. Bountiful age of free radicals actuates lipid peroxidation, which is the significant results of oxidative pressure (Erejuwa et al., 2013). Consumption of endogenous antioxidants and increase lipid peroxidation by age of free radicals may initiate the beginning phase of apoptosis in cancer (Kern and Kehrer, 2005). In our examination, we watched expanded ROS levels and diminished antioxidant status in baicalein treated cells in a focus subordinate way. Under cancerous conditions and the nearness of redox-dynamic progress metals, flavonoid mixes like baicalein can go about as favourable to the oxidant through various responses by including in redox-cycling and advancing the age of hydroxyl radicals. Further, these flavonoid mixes structure a labile aroxil radical, or a labile redox complex with a metal cation. This aroxil radical can respond with oxygen, bringing about the development of $O_2^{\cdot-}$. In this manner, exorbitantly produced free radicals harms cell compartments lead to the development of lipid peroxidation and DNA harm and are related with a reduction in antioxidants status, therefore, oxidative pressure at long last cell passing (Yoshida et al., 2013). Also, Bevara et al. (2018) announced that baicalein incites apoptosis in human colorectal carcinoma cells through ROS age and enactment of apoptotic atoms. A few flavonoid

operators, for example, resveratrol, gallic corrosive, caffeic corrosive, capsaicin and carnosol have been appeared to cause expanded cell ROS age as a ruse of especially murdering cancer cells.

Taking everything into account, we recommend that baicalein represses cell proliferation in KB-cells through ROS dependent mitochondrial interceded apoptosis as prove by the rise of ROS age bringing about oxidative DNA harm, and atomic fracture. Further, the master oxidant job of baicalein adjusts the apoptotic protein articulation. These outcomes demonstrated that baicalein could be utilized as a novel restorative operator for the clinical treatment as well as avoidance of oral cancer.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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