Global SciTech Ocean

Current Medical and Drug Research

(Contents available at www.globalscitechocean.com)

Research article

Inhibition of human neuroblastoma cells through ROS-activation by naringin

A. Arthi[1], Vijay Lobo[2], R. Vidhyavathi[2], E. Mohan Raj[2] and Arun Kumar Ramu[2]*

[1]Department of Biochemistry, PRIST Deemed University, Vallam, Thanjavur, Tamil Nadu, India
[2]Department of Biochemistry, Centre of Research and Development (CRD), PRIST Deemed University, Vallam, Thanjavur, Tamil Nadu, India.

*Corresponding author. E-mail: arunkumarkarthiphd@gmail.com

Article history

Received : July 28, 2020
Accepted : August 16, 2020

Keywords

MTT
Naringin
Neuroblastoma
ROS
SK-N-MC

ABSTRACT

Current remedial options for recurrent neuroblastoma have poor outcomes that warrant the development of novel restorative methodologies. Naringin (4',5,7-trihydroxy-flavanone-7-rhamnoglucoside), a naturally occurring flavonoid present in various Indian medicinal spices, has been shown anti-inflammatory and anticancer activities. In this paper, naringin was used to evaluate its anti-proliferative impact on human SK-N-MC neuroblastoma cell lines. Cytotoxicity and reactive oxygen species (ROS) were measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) test, respectively. It was found that naringin induced 100% cancer cell inhibition at 120 µM, hence, 30, 45 and 60 µM doses were chosen for anticancer examinations. Significant apoptosis was recorded at 60 µM dose of naringin which was found associated with the generation of ROS. Overall, the investigation indicated that naringin induces apoptosis in SK-N-MC cells to produce ROS in a mitochondria-dependent and independent manner. In conclusion, at high doses, naringin adequately inhibits the growth of solid neuroblastoma tumour and has high bioavailability, particular toxicity and a high margin of security, making it a possible candidate for a potential clinical treatment of neuroblastoma.

INTRODUCTION

Neuroblastoma is a pediatric tumour that begins from the embryonal forerunner cells of the thoughtful nervous framework. In spite of broad treatment, youngsters with high stage neuroblastoma have a helpless visualization with 20-40%. Genomic deviations in qualities legitimately engaged with apoptotic flagging are uncommon also, the middle time of neuroblastoma patients at a conclusion is a year and a half, and 40% of cases are analyzed before 1 year old enough (London et al., 2005). Practically 50% of the cases are classified as high hazard within general endurance rate under 40%. Despite sensational accelerations in the force of treatment were given, just unobtrusive improvement has been indicated resistance (Aravindan et al., 2019). Nonetheless, it is a forceful malignancy with around 70% of high-hazard patients giving far off metastasis at the hour of analysis (Ara and DeClerck, 2006). High-chance neuroblastomas are quickly dynamic and backslide is normal with presently no known healing treatment. The standard treatment choices for this malignancy incorporate medical procedure, chemotherapy, radiation treatment, bone marrow relocate, and biologic treatment. Neuroblastomas show remarkable clinical and organic heterogeneity and notwithstanding concentrated treatment, the death rate stays over half (Thole et al., 2020). The reactions of standard treatment can cause intense and long haul harm and harmfulness in different organic capacities and can prompt the advancement of optional tumours (Whittle et al., 2017). Subsequently, there is an earnest need in recognizing novel procedures in neuroblastoma that improve result with less poisonousness. In this way, more potential specialists should be recognized for neuroblastoma treatment. in neuroblastoma. Deregulation is by all accounts brought about by epigenetic occasions.

Chemotherapy is the utilization of synthetic substances or drugs to execute malignant growth cells, and its belongings are fundamental (Bregni et al., 2020). Up until this point, there are a few distinct classes of anticancer drugs dependent on their components of activity. Although the disease explicitness of focused specialists contrasted with customary chemotherapy drugs isn't outright, a considerable lot of the planned sub-atomic targets

are explicitly overexpressed or hyperactivated in malignant growth cells and advance malignant growth cell multiplication, endurance, angiogenesis and attack. Explicit specialists utilized to repress the key atoms of these pathways ordinarily are little particle inhibitors or monoclonal antibodies against proteins, for example, receptor tyrosine kinases (RTKs), or different proteins with synergist exercises. Handy abuse of more than three many years of fundamental cell flagging examination has created a rush of new mixes focusing on malignancy applicable biomolecules. Clinical assessment of these new specialists has not just uncovered in certain occurrences outstanding movement against already exceptionally hard-headed malignancies.

The flavonoids have aroused considerable intrigue as of late due to their potential gainful consequences for human wellbeing; they have been reported to have antiviral, anti-allergic, antiplatelet, calming, antitumor, and antioxidant exercises (Hosseinzadeh et al., 2020). In an ongoing year study, it was found that members with the most elevated admission of flavonoids and proanthocyanidins had a lower danger of oral cancer and laryngeal cancer, separately (Romagnolo and Selmin, 2012). In addition, numerous fragmentation products of flavonoids are substrates or regulators of medication metabolizing and stress-responsive catalysts that can apply biological antioxidant impacts. Dietary flavonoid glycoside, present in citrus organic products, demonstrates antioxidant and anticancer properties and uncovered that didymin was equipped for forestalling phthalate ester-associated cancer aggravation. Here, we sought to inspect whether naringin (Fig. 1) treatment can apply against tumorigenic impacts in neuroblastoma cells.

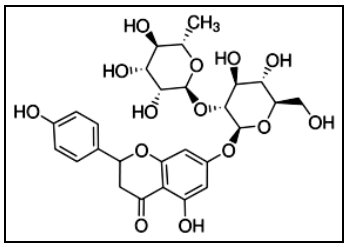

Fig. 1. Chemical structure of naringin

MATERIALS AND METHODS

Chemicals

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. Naringin was kindly gifted by Dr Bakrudeen Ali Ahmed Abdul, Faculty of Applied Science, Ton Duc Thang University, Ho Chi Minh, Vietnam.

Cell culture

The present work was carried out in SK-N-MC neuroblastoma cell line, obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were cultured as a monolayer in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), penicillin and streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Cells were grown in 75cm^2 tissue culture flasks and used for experiments when in an exponential growth phase.

Cell proliferation assay

The effect of naringin on the cell proliferation of SK-N-MC was determined by MTT assay based on the detection of mitochondrial dehydrogenase activity in healthy cells. SK-N-MC 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 naringin. After 24 h, the cells were incubated with 100ml 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 Readwell touch, ELISA plate reader (Robonic, India).

Determination of intracellular ROS levels

Intracellular ROS level was measured using a non-fluorescent probe, 2,7-diacetyl dichloro-fluorescein (DCFH-DA), that can penetrate into the intracellular matrix of cells where it is oxidized by ROS to fluorescent dichlorofluorescein (DCF). Naringin-treated SK-N-MC cells were seeded in 6 well plates (2×10^{6}cells/well) and incubated with 10 M DCFH-DA for 30 min at 37 °C. Fluorescent measurements were made with excitation and emission filters set at 485 ± 10 nm and 530 ± 12.5 nm, respectively (Shimadzu RF-5301 PC spectrofluorometer). The cells were also observed under a fluorescence microscope using blue filter (450-490 nm) (Nikon, Eclipse TS100, Japan).

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 statistical significance of the difference between control and treated groups.

RESULTS

Cytotoxicity study

The cytotoxic effect of naringin on SK-N-MC cells was determined by MTT assay (Table 1). Cells were treated with different concentrations of naringin (5-150 µM) for 24 h incubation, which revealed a dose-dependent inhibition of cell proliferation. Maximum cell death was observed at 120 µM concentrations. Hence, the 50% inhibitory concentration (IC_{50}) of naringin for SK-N-MC cells at 60 µM apparent from growth inhibition curve. We selected 30, 45 and 60 µM doses of naringin for further studies.

Table 1. Inhibitory effect of naringin on SK-N-MC cells using MTT assay

Replication	Control	30 µM	45 µM	60 µM
1	65.2 ± 5.3	32.7 ± 3.4	27.3 ± 2.5	19.5 ± 1.5
2	64.5 ± 5.1	31.8 ± 3.0	24.8 ± 2.1	18.5 ± 1.3
3	59.0 ± 4.7	32.0 ± 3.1	25.0 ± 1.9	17.2 ± 1.2

Data were expressed as Mean ± SE.

Effect on the generation of intracellular ROS

The intracellular ROS generation was measured by DCFH-DA staining. Figure 2 illustrates the levels of ROS generation in control and naringin treated cells. SK-N-MC cells were treated with different concentration of naringin (30, 45 and 60 µM) shows significantly increased levels of ROS generation which indicating extreme green fluorescence intensity as compared to untreated control cells.

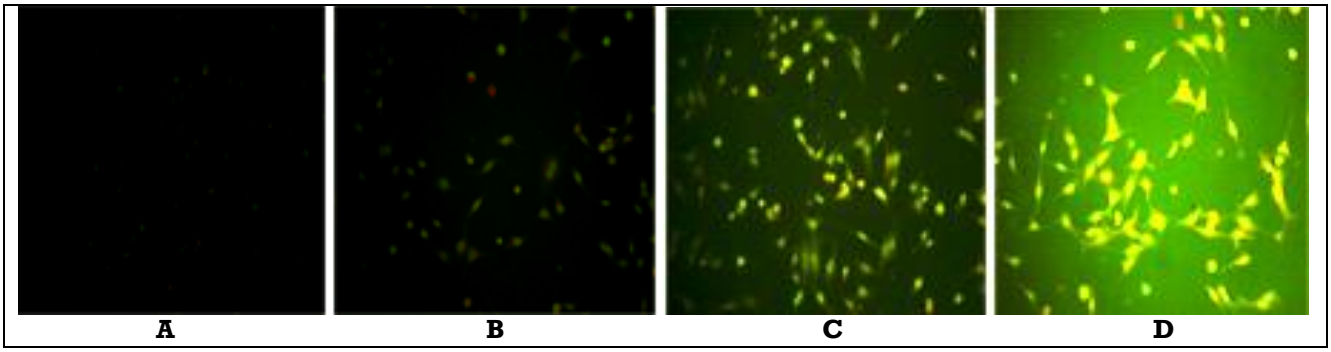

Fig. 2. Effect of naringin on intracellular ROS generation in SK-N-MC cells using DCFH-DA staining. Photo micrographic image of A: Control cell shows weak fluorescence DCF; B: Treatment with 30 µM shows mild fluorescence; C: Treatment with 45 µM shows moderate fluorescence; D: Treatment with 60 µM showed highest DCF fluorescence indicating increased ROS generation.

DISCUSSION

Neurological disorders have been linked to raised degrees of oxidative pressure and apoptosis (Shukla et al., 2011). Oxidative pressure and free extreme generation have been shown to assume a significant role in regulating redox reactions in vivo/in vitro contributing to the generation of ROS (Snezhkina et al., 2019). A few pieces of evidence indicate that oxidative pressure also assumes a major role in modulating the biochemical changes resulting in ageing and neurodegenerative disorders (Kim et al., 2015). Anti-oxidants are exogenous or endogenous molecules which smother the oxidative worry by neutralizing ROS and other free radicals, subsequently exhibiting their helpful potential.

In the present investigation, we observed the cytotoxic impact of H_2O_2 just as the protective impact of naringin by MTT measures. Our findings were additionally supported by the morphological observations. The cell reasonability test uncovered that SK-N-MC cells pretreated with naringin were like the untreated control cells. Evaluation of neuronal cytotoxicity induced by ROS in SK-N-MC cells uncovered a significant cell passing that was neutralized by naringin. Protective role of naringin was additionally confirmed by MTT examine. Our current findings are in line with the various report. ROS promptly penetrates into cells and generates exceptionally receptive hydroxyl radicals that progressively assault cell components including lipids, protein, and DNA in this way inducing oxidative harms (Kryston et al., 2011). The generation of ROS was quantified using the fluorescent probe DCFHDA. DCFH is exceptionally sensitive to a few ROS and can be oxidized to a profoundly fluorescent 2′,7′-dichlorofluorescein (DCF) (Kalyanaraman et al., 2012). The DCF fluorescence indicates the resultant oxidative worry because of overproduction of ROS or the depletion of antioxidants without any identification

of explicit ROS. We observed increased ROS production in the SK-N-MC cells pretreated with naringin. Flavanone has several phenolic hydroxyl groups, which are generally credited to their powerful antioxidant properties (Yao et al., 2006).

Mitochondrial dysfunction and oxidative pressure have been embroiled in the physiology and pathogenesis of many maladies; therefore, the mitochondrial membrane potential change induced by ROS. Our outcomes demonstrating the inhibitory impact of naringin in the cells treated with ROS increased the integrity of the mitochondrial membrane, however, cells pretreated with naringin inhibitory consequences for the loss of mitochondrial membrane integrity.

CONCLUSION

In conclusion, treatment with naringin inhibits SK-N-MC cells through antioxidant and upregulation. This may provide an experimental platform for additional examinations in human cell lines and use in clinical settings. Therefore, SK-N-MC as a potential candidate could be read for its helpful potential and development of preventive remedy for a few human neuroblastoma maladies.

CONFLICTS OF INTEREST

The author declares no conflicts of interest

REFERENCE

Ara T, DeClerck YA (2006). Mechanisms of invasion and metastasis in human neuroblastoma. Cancer and Metastasis Reviews, 25, 645-657.

Aravindan N, Jain D, Somasundaram DB, Herman TS, Aravindan S (2019). Cancer stem cells in neuroblastoma therapy resistance. Cancer Drug Resistance, 19, 948-967.

Bregni G, Akin Telli T, Camera S, Deleporte A, Moretti L, Bali AM, Liberale G, Holbrechts S, Hendlisz A, Sclafani F (2020). Adjuvant chemotherapy for rectal cancer: Current evidence and recommendations for clinical practice. Cancer Treatment Reviews, 83, 101948.

Hosseinzadeh E, Hassanzadeh A, Marofi F, Alivand MR, Solali S (2020). Flavonoid-Based Cancer Therapy: An Updated Review. Anti-Cancer Agents in Medicinal Chemistry, doi. 10.2174/1871520620666200423071759.

Kalyanaraman B, Darley-Usmar V, Davies KJ, Dennery PA, Forman HJ, Grisham MB, Mann GE, Moore K, Roberts LJ, Ischiropoulos H (2012). Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. Free Radical Biology and Medicine, 52, 1-6.

Kim GH, Kim JE, Rhie SJ, Yoon S (2015). The role of oxidative stress in neurodegenerative diseases. Experimental Neurobiology, 24, 325-340.

Kryston TB, Georgiev AB, Pissis P, Georgakilas AG (2011). Role of oxidative stress and DNA damage in human carcinogenesis. Mutation Research, 3, 193-201.

London WB, Boni L, Simon T, Berthold F, Twist C, Schmidt ML, Castleberry RP, Matthay KK, Cohn SL, De Bernardi B (2005). The role of age in neuroblastoma risk stratification: the German, Italian, and children's oncology group perspectives. Cancer Letters, 18, 257-266.

Romagnolo DF, Selmin OL (2012). Flavonoids and cancer prevention: a review of the evidence. Journal of Nutrition in Gerontology and Geriatrics, 31, 206-238.

Snezhkina AV, Kudryavtseva AV, Kardymon OL, Savvateeva MV, Melnikova NV, Krasnov GS, Dmitriev AA (2019). ROS Generation and Antioxidant Defense Systems in Normal and Malignant Cells. Oxidative Medicine and Cellular Longevity, 2019, 6175804.

Thole TM, Toedling J, Sprussel A, Pfeil S, Savelyeva L, Capper D, Messerschmidt C, Beule D, Groeneveld-Krentz S, Eckert C, Gambara G, Henssen AG, Finkler S, Schulte J. H, Sieber A, Bluethgen N, Regenbrecht CRA, Kunkele A, Lodrini M, Eggert A, Deubzer HE (2020). Reflection of neuroblastoma intratumor heterogeneity in the new OHC-NB1 disease model. International Journal of Cancer, 15, 1031-1041.

Whittle SB, Smith V, Doherty E, Zhao S, McCarty S, Zage PE (2017). Overview and recent advances in the treatment of neuroblastoma. Expert Review of Anticancer Therapy, 17, 369-386.

Yao H, Liao ZX, Wu Q, Lei GQ, Liu ZJ, Chen DF, Chen JK, Zhou TS (2006). Antioxidative flavanone glycosides from the branches and leaves of *Viscum coloratum*. Chemical and Pharmaceutical Bulletin, 54, 133-135.

How to cite this article?

Arthi A, Lobo V, Vidhyavathi R, Mohan Raj E, Ramu AK (2020). Inhibition of human neuroblastoma cells through ROS-activation by naringin. Current Medical and Drug Research, 4 (1), Article ID 203.

*****