Indian Journal of Experimental Biology
Vol. 62, March 2024, pp. 187-191
DOI: 10.56042/ijeb.v62i03.803

Senolytic effects of berberine protects fibroblasts cells against UV induced ageing

Athira Raji Sunilkumar[1], Blessan Vijayakumar Anitha[1], Karthika Saju Shylaja[1], Neethu Bindhu[1], Niranjana Ajayakumar Sheeja[1], Lekshmi Vijayanathan[1], Nithin Vijayakumar[2] & Rajesh Ramachandran[2]*

[1]Department of Biochemistry, Emmanuel College, Vazhichal, Thiruvananthapuram, Kerala, India

[2]Department of Molecular Biology, Center For Research on Molecular and Applied Sciences (P) Ltd., Thiruvananthapuram, Kerala, India

Received 09 May 2023; *revised 12 January 2024*

The gradual and irreversible pathophysiological process of ageing escalates many chronic diseases which may lead to disability and ultimately death in elderly individuals. Premature ageing is largely associated with DNA damages similar to the ones associated with UV-induced photoaging and the protective effects of phytochemicals as inhibitors of cellular senescence is an area of increased research significance. In the present study, we tried to determine the UV protective and senolytic effects of berberine in experimental ageing models. L929 cells were subjected to UV treated and treated with different concentrations of berberine from 3.1 to 25 µg.mL^{-1}. The effect of berberine on intracellular reactive oxygen species (ROS) generation was determined by 2'-7'-dichlorodihydrofluorescein diacetate (DCFDA) staining. The DNA damages were measured by comet assay and senescence was determined using galactosidase staining. The methylthiazolyldiphenyl-tetrazolium bromide (MTT) results confirmed the berberine-associated protection in L929 cells with significant improvement in cellular morphology. UV exposure significantly increased ROS generation and was decreased in groups co-treated with berberine. UV irradiation resulted in increased tail length whereas co-treatment with berberine significantly reduced DNA damage. X gal staining confirmed the senolytic effects of berberine. The experimental findings confirm that berberine can limit the oxidative injury and DNA damages produced by UV rays which inturn find applications in limiting ageing and associated metabolic diseases.

Keywords: Comet assay, DNA damage, Photoaging, Photochemoprotection, Radiation, Reactive oxygen species (ROS), Senescence, Ultraviolet rays

Photoaging or photo carcinogenesis occurs due to excessive solar ultraviolet radiation altering DNA, cellular antioxidant balance, signal transduction pathways, immunology and the extracellular matrix (ECM) is a serious threat to human health[1]. The most common DNA modifications and alterations imparted by UV radiation includes thymine-thymine dimer formation and loss of tumor suppressor gene p53, etc. Another impact is that increased UV radiation generates reactive oxygen species (ROS), reducing the cellular antioxidant status thereby prompting oxidative stress. As reviewed, the increase in oxidative stress alters signal transduction pathways, such as mitogen-activated protein kinase (MAPK), nuclear factorkappa beta (NF-κB)/p65, janus kinase (JAK), signal transduction and activation of transcription (STAT) and the nuclear factor erythroid 2-related factor 2 (Nrf2)[2,3]. In addition, UV radiation induces proinflammatory genes and causes immunosuppression by depleting the number and activity of the epidermal Langerhans cells[3]. These deleterious effects of UV rays are scientifically proven to contribute to premature ageing and pathological conditions including melanoma.

Several approaches are employed to alleviate the consequences of excess UV exposure and photoaging, the process termed as 'photochemoprotection'. The role of antioxidant is quite distinct in the process.In accordance with recent findings it is reported that exogenous antioxidant supplementation or antioxidant based lotions can be a potent strategy to combat photoaging and pathologies[4].

The isoquinoline alkaloid berberine is found in many species like *Coptis* sp., *Berberis* sp., *Phellodendron* sp. Etc., is widely used in traditional medicinal systems including Chinese medicinal system[5,6]. Numerous studies also already depicted the diverse pharma-cological properties of berberine including antioxidant, anti-inflammatory, antiproliferative, antihypertensive, neuroprotective, etc.[7-10]. Studies point to the protective

*Correspondence:
Phone: +91 9895349416 (Mob.)
E-Mail: info@crmas.in

role of berberine on cardiac senescence by modulating Klotho/SIRT1 pathway[11]; on retinal pigment epithelium by interrupting ROS-DDR feedback loop thereby downregulating REDD1[12]. The recent studies have suggested anti-ageing properties of berberine but its effects on cellular senescence and DNA damages remains unexplored.

Senescence associated with DNA damages due to UV exposure produce intracellular signalling leading to mitochondrial dysfunction and aggravating cell death[13]. Strategies to remove senescent cells can in turn be useful as a pharmacological target for alleviating geriatric and chronic diseases associated with fundamental ageing. In the present study, we have investigated the UV protective and senolytic effects of berberine in experimental ageing models.

Materials and Methods

Materials and chemical reagents

L929 (murine fibroblast cells) were used as the *in vitro* model for the studies, which was purchased from National centre for cell science (NCCS), Pune, India and maintained in Dulbecco's modified Eagles medium (DMEM) (gibco, Thermo Fisher Scientifics; Lot No: 2518079). Trypsin (gibco, Thermo Fisher Scientifics; Cat No: 25300062), methylthiazolyldiphenyl-tetrazolium bromide (MTT) (HiMedia; Lot No: MB186-500MG), 2'-7'-dichlorodihydrofluoresceindiacetate (DCFH-DA) (Sigma-Aldrich; Lot No: 059M4133V), 5-bromo-4-chloro-3-indolyl β-d-galactopyranoside (X-gal) (Sigma-Aldrich; Cas No: 7240-90-6). HPLC grade berberine was procured from Sigma – Aldrich (Sigma-Aldrich; Cas No: 633-65-8).

UV irradiation

For irradiation of UV, L929 cells were seeded into 96-well plates (NUNC) with a cell suspension of 3×10^4 cells/ well and incubated at 37°C in a humidified CO_2 incubator (5%) (NBS Eppendorf, Germany)[14]. The UV irradiation was carried out for 30 min using a Philips UV lamp.

In vitro cytoprotective effect of berberine on UV exposed L929 cells

Inorder to assess the cytoprotective effect of berberine on UV exposed L929 cells, MTT assay was performed as per Mosmann[15] and Jerard *et al.*[16]. The UV exposed L929 cells were co-treated with varied concentrations (3.1, 6.25, 12.5 and 25 $\mu g.mL^{-1}$) of berberine and incubated for 24 h. Followed by incubation, the medium was removed, and the cells were added with 50 µL of MTT solution (5 $mg.mL^{-1}$) and incubated at 37°C for 3 h. After incubation, the cells were washed using sterile PBS, treated with DMSO (200 µL) incubated at 37°C for 30 min. Thus, the obtained solution was centrifuged (2 min, 4°C, 4000 rpm) (Eppendorf) and the absorbance was read at 540 nm (ELISA plate reader) keeping DMSO as blank to calculate the percentage viability and the protective concentration of berberine was determined.

Detection of intracellular Reactive Oxygen Species (ROS) by DCFH-DA staining

L929 cells were seeded onto 96-well plate at a density of 3×10^4 cells/well and allowed to stand for 24 h at 37°C in a humidified CO_2 incubator (5%). UV exposed cells were kept as positive control and untreated wells remained as negative control. Followed by the incubation of 24 h, the cells were washed with PBS and treated with (100 µM DCFH-DA) for 1 h at 37°C in a humidified CO_2 incubator as per Ramachandran & Saraswathy[17]. The fluorescence obtained was imaged on Olympus CKX41 connected with Optika Pro5 camera and the fluorescence was quantitated using Qubit™ 3 Fluorometer, Thermo Fischer Scientific.

Detection of genoprotective effect of berberine by Comet assay

The genoprotective efficacy was determined in UV irradiated L929 cells co-administrated with 3.1 $\mu g.mL^{-1}$ of berberine by comet assay (Single cell gel electrophoresis). After performing comet assay, each slides were photographed with an aid of an inverted epi-fluorescent microscope (Olympus CKX41 connected with Optika Pro5 camera). The photomicrographs were subjected to measure different parameters such as comet length, tail length and olive moment and documented as mentioned by Ramachandran & Saraswathi[18].

X-gal staining

For the determination of senescence X-gal staining was performed. The cells were cultured as for different treatments and kept for incubation. Followed by incubation of 24 h, the medium was removed and washed with sterile PBS twice. The cells were then fixed with 4% formaldehyde and added with 500 µL of X-gal (1 $mg.mL^{-1}$ of PBS), kept for incubation at 37°C in a humidified CO_2 incubator. The cells were then photographed with Olympus CKX41 connected with Optika Pro5 camera and the micrographic observations were kept as images.

Statistical analysis

The results were expressed as the mean ± standard deviation of three independent experiments. The data

were further analyzed using One-way analysis of variance (ANOVA), followed by Dunnets post hoc test. Values of P <0.05 were considered as statistically significant. The statistical analyses were performed using Graph pad Prism 5 software.

Results

In vitro cytoprotective effect of berberine on UV exposed L929 cells

The cytoprotective effect of berberine was tested by a colorimetric method, MTT assay. Based on the MTT assay, the cytoprotective effect of berberine was expressed in terms of cell viability after 24 h of incubation. In the L929 cells, irradiated with UV, there occurs a significant reduction in cell viability of 50.48±1.92%. Based on the obtained results, it was concluded that 3.1 µg.mL^{-1} of berberine (95.19±2.82% of percentage viability) showed significant protective effects on UV irradiated L929 cells (Figs 1 & 2).

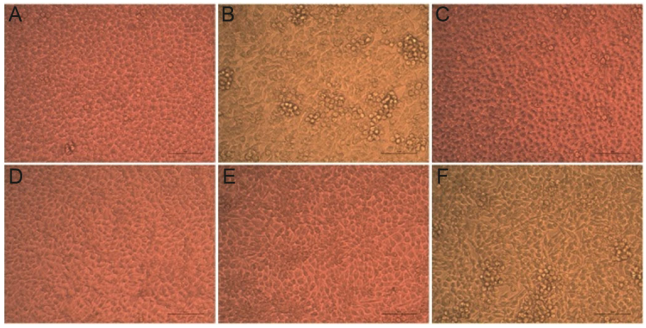

Fig. 1 — Photomicrographs depicting the effect of berberine co-administration on cultured L929 cells exposed to UV. (A) Untreated control cells; (B) UV exposed cells; and (C-F) UV exposed cells co-administrated with varied concentrations of berberine 3.1, 6.25, 12.5, and 25 µg, respectively, n=3

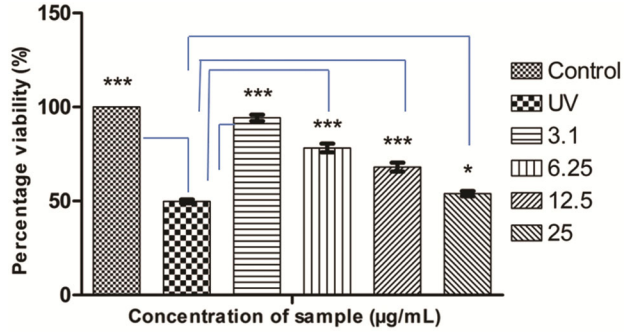

Fig. 2 — Graphical representation depicting the cytoprotective effect of berberine co-administration on UV irradiated L929 cells by MTT assay. [Along Y axis percentage viability and along X axis varied concentration of berberine. All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnets test were performed to analyse data. *P <0.1 and **P <0.001 compared to UV irradiated groups, n=3

Effect of berberine on ROS generation induced by UV irradiation

In the present study, untreated control cells, UV irradiated cells and UV irradiated cells treated with berberine was checked for the ROS generation. By measuring the fluorescence emitted using fluorometer, it was observed that in the UV irradiated cells, it is showing a higher fluorescence intensity of 3125.5±16.96 AU while comparing with the untreated control (316.5±8.05); whereas in case of the berberine co-administration, it is decreased significantly (1179.90±23.10) (Figs 3 & 4).

Berberine reduces DNA damages induced by UV radiation

The level of nuclear DNA damage was determined by measuring the different parameters following the comet assay under high alkaline conditions, which reflects the extent of DNA breakage in both the single and double strand breaks. From the obtained results it is very much clear that there was no DNA damage, because of the absence of comet formation (Comet length = 23.08±0.10 px; Tail length = 1.025±0.39 px) whereas in the UV irradiated cells there occurs a significant DNA damage and comets were formed (Comet length = 85.47±0.59 px; Tail length = 55.86±0.91 px) (Table 1). In the berberine co-administration the level of DNA damage was

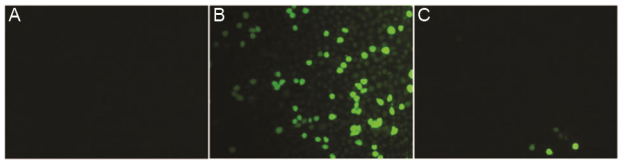

Fig. 3 — Determination of ROS generation by DCFDA staining method. (A) untreated control cells; (B) L929 cells irradiated with UV; and (C) L929 cells irradiated with UV co-administrated with berberine, n=3.

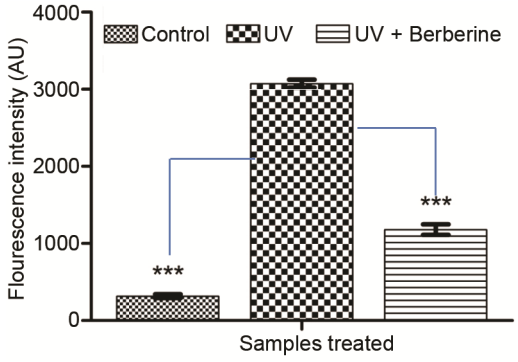

Fig. 4 — Graphical representation depicting the ROS generation by DCFH-DA method using Qubit™ 3 Fluorometer, Thermo Fischer Scientific. [Along Y axis fluorescence intensity in terms of arbitrary units (AU) and along X axis – samples treated. All experiments were done in triplicates and results represented as Mean +/- SE. One-way ANOVA and Dunnets test were performed to analyse data. *P <0.001 compared to UV irradiated groups

Table 1 — Comet assay showing DNA damage in UV exposed murine fibroblast cells (L929) and protection by berberine

Samples	Comet length (px)	Tail length (px)	Olive movement (px)
Control	23.08±0.10	1.025±0.39	0.214±0.45
UV	85.47±0.59	55.86±0.91	31.25±0.53
UV + Ber	25.68±076	32.25±0.86	10.23±0.83

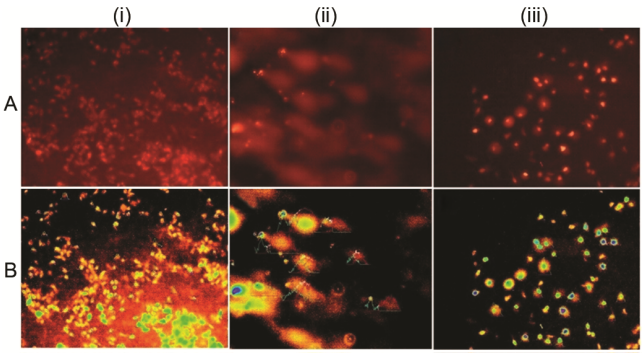

Fig. 5 — Comet assay for determination of geneoprotective effect of berberine using single cell electrophoresis, n=3. (A) micro-photoghraphs: and (B) scored microphotoghraphs of comet images.

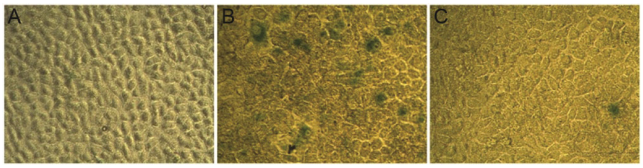

Fig. 6 — X-gal staining (A) untreated control cells; (B) L929 cells irradiated with UV; and (C) L929 cells exposed to UV irradiation co-administrated with berberine, n=3.

significantly decreased while comparing with the UV irradiated cells and which substantiated the cytoprotective effect of berberine (Fig. 5).

X-gal staining

For determining senescence, Senescence Associated β-galactosidase (SA-β-gal), a lysosomal enzyme was employed. Senescence is measured by *in situ* staining using the X-gal a chromogenic substrate which turns blue in the presence of β-galactosidase. From the results it can be confirmed that the cultured L929 cells exposed to UV irradiation an increase in blue stained senescent cells was observed. The numbers of senescent cells are more in UV exposed cells while comparing with the untreated control cells. The co-administration of berberine has reduced blue stained senescent cells significantly (Fig. 6).

Discussion

The major aim of the present study was to determine the antiaging and anti-UV effects of phytochemical berberine on L929 fibroblast cell line model. Development of a potent antisenolytic drugs using phytochemcials can find applications in treatment of geriatric and ageing associated diseases. In this context, here in the present study, we determined the effects of berberine as anti-ageing to limit the UV induced DNA damages in cells. The cells were treated with berberine at different concentrations and were exposed to UV for 30 min. UV alone exposed cells were kept as negative controls. As per our findings it can be observed that UV reduced the cell viability to 52% whereas co treatment with 3.1 ug/mL of berberine has attenuated the UV induced damages and increased the cell viability to 98%. The concentrations for efficacy studies were selected based on cytotoxicity studies and the concentrations retained cell viability more than 70% was selected for cytoprotection studies. All the experiments were done for 24 h as ADMET data's of berberine shows a quick metabolic clearance within 24 h which prompted us to use 24 h for the screening studies. The results confirmed UV protective effects of berberine which was found to be decreased in higher concentrations.

The decrease in cell viability following UV exposure can be inturn a result of increased ROS generation and in this context we measured the pro oxidant effects of different UV radiations in L929 cells as per standard procedures of DCFDA staining. The findings portrays more ROS generation (nearly 30 fold increase) in groups exposed to UVA radiation whereas co treatment with berberine significantly reduced the ROS generation. These supports the reports that generation of ROS by UV radiation is one of the mechanisms through which UV light can manifest its possible detrimental effects on health. When an imbalance develops due to ROS generation exceeding the body's antioxidant defence mechanisms, oxidative stress can develop19 and antioxidant compounds can be beneficial in preventing increased ROS generation.

The genoprotective effects of berberine over the DNA damages induced UV rays were further confirmed by comet assay. The comet assay or single cell gel electrophoresis is a versatile, reliable, cost-efficient, and fast technique for detecting DNA damage and repair in any tissue. It is useable in almost any cell type and applicable to both eukaryotic and prokaryotic organisms. Our results show an increase in comet length and tail length in groups exposed to UV rays. This confirms that three to four fold increase in comet length and nearly 50 fold

increase in tail length. Co treatment with berberine effectively reduced the comet length and tail length which clearly depicts the protective effects of berberine. This finding is in accordance with Sweetman *et al*.[20] who have reported that antioxidants can reduce the DNA damages produced by ROS generation.

Conclusion

The above findings suggest that berberine can limit the oxidative injury and DNA damages produced by UV rays and thereby find applications in limiting ageing and associated metabolic diseases. Currently, prevention and treatment strategies for photoaged skin mostly center on strengthening antioxidant defense for the cells, and these experimental findings confirm the antiaging potential of berberine which can find applications in therapeutic regimens.

References

1 Hong JA, Bae D, Oh KN, Oh DR, Kim Y, Kim Y, Jeong Im S, Choi Ej, Lee Sg, Kim M, Jeong C & Choi CY, Protective effects of Quercus acuta Thunb. fruit extract against UVB-induced photoaging through ERK/AP-1 signaling modulation in human keratinocytes. *BMC Complement Med Therap*, 22 (2022) 6.

2 Gromkowska-Kępka KJ, Puścion-Jakubik A, Markiewicz-Żukowska R & Socha K, The impact of ultraviolet radiation on skin photoaging — review of *in vitro* studies. *J Cosmet Dermatol*, 20 (2021) 3427.

3 Bosch R, Philips N, Suárez-Pérez JA, Juarranz A, Devmurari A, Chalensouk-Khaosaat J & González S, Mechanisms of Photoaging and Cutaneous Photocarcinogenesis, and Photoprotective Strategies with Phytochemicals. *Antioxidants*, 4 (2015) 248.

4 Petruk G, Giudice RD, Rigano MM & Monti MD, Antioxidants from Plants Protect against Skin Photoaging. *Oxid Med Cell Longev*, 2018 (2018) 1454936.

5 Ai X, Yu P, Peng L, Luo L, Liu J, Li S, Lai X, Luan F & Meng X, Berberine: A Review of its Pharmacokinetics Properties and Therapeutic Potentials in Diverse Vascular Diseases. *Front Pharmacol*, 12 (2021) 762654.

6 Yao J, Wei W, Wen J, Cao Y & Li H, The efficacy and mechanism of berberine in improving aging-related cognitive dysfunction: A study based on network pharmacology. *Front Neurosci*, 17 (2023) 1093180.

7 Jeong HW, Hsu KC, Lee JW, Ham M, Huh JY, Shin HJ, Kim WS & Kim JB, Berberine suppresses proinflammatory responses through AMPK activation in macrophages. *Am J Physiol Endocrinol Metab*, 296 (2009) E955.

8 Pirillo A & Catapano AL, Berberine, a plant alkaloid with lipid- and glucose-lowering properties: from *in vitro evidence to clinical studies. Atherosclerosis*, 243 (2015) 449.

9 Xu Z, Feng W, Shen Q, Yu N, Yu K, Wang S, Chen Z, Shioda S & Guo Y, Rhizoma coptidis and berberine as a natural drug to combat aging and aging-related diseases via anti-oxidation and AMPK activation. *Aging Dis*, 8 (2017) 760.

10 Dang Y, An Y, He J, Huang B, Zhu J, Gao M, Zhang S, Wang X, Yang B & Xie Z, Berberine ameliorates cellular senescence and extends the lifespan of mice via regulating p16 and cyclin protein expression. *Aging Cell*, 19 (2020) e13060.

11 Cong Li, Shuang Jiang, Hengfei Wang, Yuhong Wang, Yanxing Han & Jiandong Jiang, Berberine exerts protective effects on cardiac senescence by regulating the Klotho/SIRT1 signaling pathway. *Biomed Pharmacother*, 151 (2022) 113097.

12 Qingqiu Chen, Guang Xin, Shiyi Li, Yuman Dong, Xiuxian Yu, Chengyu Wan, Zeliang Wei, Yuda Zhu, Kun Zhang, Yilan Wang, Fan Li, Cuicui Zhang, E Wen, Yulong Li, Hai Niu & Wen Huang, Berberine-mediated REDD1 down-regulation ameliorates senescence of retinal pigment epithelium by interrupting the ROS-DDR positive feedback loop. *Phytomedicine*, 104 (2022) 154181.

13 Gasek NS, Kuchel GA, Kirkland JL & Xu M, Strategies for targeting senescent cells in human disease. *Nat Aging*,1 (2021) 870.

14 Ribeiro FM, Volpato H, Lazarin-Bidóia D, Desoti VC, de Souza RO, Fonseca MJV, Ueda-Nakamura T, Nakamura CV & Silva SO, The extended production of UV-induced reactive oxygen species in L929 fibroblasts is attenuated by posttreatment with Arrabidaea chica through scavenging mechanisms. *J Photochem Photobiol B*, 178 (2018) 175.

15 Mosmann T, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*, 65 (1983) 55.

16 Jerard C, Michael BP, Chenicheri S, Vijayakumar N & Ramachandran R, Rosmarinic Acid-Rich Fraction from Mentha arvensis Synchronizes Bcl/Bax Expression and Induces Go/G1 Arrest in Hepatocarcinoma Cells. *Proc Natl Acad Sci, India, Sect B Biol Sci*, 90 (2019) 515.

17 Ramachandran R & Saraswathy M, Up-regulation of nuclear related factor 2 (NRF2) and antioxidant responsive elements by metformin protects hepatocytes against the acetaminophen toxicity. *Toxicol Res*, 3 (2014) 350.

18 Ramachandran R, Saraswathi M. Postconditioning with metformin attenuates apoptotic events in cardiomyoblasts associated with ischemic reperfusion injury. *Cardiovasc Ther*, 35 (2017).

19 Jager J, Putnick DL & Bornstein MH, II. More than just convenient: the scientific merits of homogeneous convenience samples: developmental methodology. Monographs Society *Res Child*, 82 (2017) 13.

20 Sweetman SF, Strain JJ & McKelvey-Martin VJ, Effect of antioxidant vitamin supplementation on DNA damage and repair in human lymphoblastoid cells. *Nutr Cancer*, 27 (1997) 122.