Indian Journal of Natural Products and Resources
Vol. 1(4), December 2010, pp. 462-465

Inhibition of UV induced adversaries by β-glucogallin from Amla (*Emblica officinalis* Gaertn.) fruits

Muhammed Majeed, Beena Bhat and T S Susmitha Anand*

Sami Labs Limited, 19/1 & 19/2, I main, II phase, Peenya Industrial Area, Bengaluru 560 058, Karnataka, India

Received 21 January 2010; Accepted 13 July 2010

Photoprotection efficacy of Amla (*Emblica officinalis* Gaertn.) fruit extract was investigated by various *in vitro* models. It was found that 1-O-Galloyl-β-D-glucose (β-glucogallin) active in Amla extract has a significant role in photoprotection efficacy due to its inhibitory effect on Ultraviolet irradiation (UV) induced adversaries in cell cultures. β-Glucogallin treatment at a concentration of 10 to 80 µg/ml in B16F1 mouse melanoma cells showed a dose dependent decrease of 15 to 55% in UV induced melanogenesis. β-Glucogallin treatment at a concentration of 10 to 80 µg/ml, also showed a dose dependent decrease of 30 to 90% in UV induced cytotoxicity and a decrease of 42 to 82% in UV induced Reactive Oxygen Species (ROS) generation in Swiss 3T3 mouse fibroblast cells. It showed a very high Oxygen Radical Absorbance Capacity (ORAC) value of 4200 µmol Trolox equivalents/g. Our evaluations shown that β-glucogallin from Amla extract has significant photoprotection efficacy.

Keywords: Amla fruit, Antioxidant, *Emblica officinalis*, β-Glucogallin, Photoprotection, Ultraviolet irradiation.

IPC code; Int. cl.[8] — A61K 36/47, A61K 131/00, A61P 17/18, A61Q 17/00

Introduction

Emblica officinalis Gaertn. (Family — Euphorbiaceae), commonly known in India as Amla is used in Ayurveda for its cosmetic applications due to its antioxidant properties. Earlier, the fruits of Amla had been claimed to be a rich source of Ascorbic acid and its high antioxidant potential was attributed to the presence of ascorbic acid[1]. It was later reported that low molecular hydrolysable tannins emblicanins A and B contribute to the antioxidant potential of Amla[2]. However, recent studies have confirmed that only trace amounts of Ascorbic acid are found in Amla extract and the earlier authors reported antioxidant hydrolysable tannins, emblicanins A and B, correspond to 1-O-Galloyl- β-D-glucose (β-glucogallin) and mucic acid 1,4-lactone 5-O-gallate, respectively[3]. Although Amla extract is known to reduce UV induced erythema, the component in Amla fruits that has significant role in photoprotection was not studied earlier. Due to the presence of trace amounts of Ascorbic acid in the fruits of *E. officinalis*, β-glucogallin can be the active principle which is significantly responsible for the photoprotection efficacy of Amla extract. Synthetic sunscreens are reported to cause skin cancer and on absorption through skin may also affect the immune system. Natural phytochemicals are therefore, preferred over for sunscreen applications. The present work is aimed at investigating the photoprotection efficacy of a natural chemical constituent, β-glucogallin from Amla extract, to promote the usage of a natural plant product for sunscreen applications.

*Correspondent author:
E-mail: susmitha@samilabs.com;
Phone: 91 80 28397973; Fax: 91 80 28373035

Materials and Methods

Plant material

Fresh fruits (10 kg) of Amla were procured locally from Bengaluru market. It was identified by our in-house botanist Benny Daniel, and a voucher specimen (P-80019) was deposited in the herbarium of Sami Labs Limited.

Preparation of the fruit extracts[3]

The freeze-dried fruit extract of *E. officinalis* was obtained from the Phytochemistry Department of Sami Labs Limited[3]. The fruits were washed thoroughly and shade dried. They were cut into pieces and half were expressed to get juice and the other half cold macerated with water (6l) at room temperature for 2 h with stirring. The filtered juice (2.1l) and extract were freeze-dried to get 40 and 30 g of dry powder, respectively.

Isolation of 1-O-Galloyl- β-D-glucose (β-glucogallin)[3]

β-Glucogallin was isolated from the freeze-dried extract of Amla using preparative HPLC system by the Analytical Chemistry Department of Sami Labs Limited[3]. The extract was separated into seven major fractions and lyophilized. The third fraction gave pure amorphous white powder, β-glucogallin. The NMR spectral characterization of the third fraction confirmed that the compound was β-glucogallin.

Test organisms

Swiss 3T3 mouse fibroblast cells and B16F1 mouse melanoma cells were procured from ATCC, Manassas, VA, USA. They were cultured in Dulbecco's minimum essential medium (DMEM) containing 10% Foetal bovine serum (FBS) procured from Sigma, St. Louis MO., USA.

Inhibition of UV induced cytotoxicity

Swiss 3T3 fibroblast cells were used for the assay at a seeding density of 3000 cells per well of 96 well clear bottom microplate in DMEM and incubated at 37°C in 95% air and 5% CO_2 in a CO_2 incubator for 24 hours. The cells were then treated with varying concentrations of β-glucogallin in culture medium and exposed to UV B irradiation by three G15T8E UV B lamps (Sankyo Denki Co., Ltd, Japan) having 14.7W lamp wattage, 0.3A lamp current, 55V lamp voltage, UV output of 3.1W and an intensity of 33.3 μW cm^{-2}. The cells were exposed at a distance of 30cm from the source for 7 minutes. After exposure to UV B irradiation, the sample containing medium was replaced with fresh medium without sample and incubated in CO_2 incubator for 48 hours. The cell viability was then determined by Neutral Red Uptake (NRU) staining technique[4]. Control1 cells were maintained under similar conditions with sample treatment and without UV irradiation. Control2 cells were also maintained under similar conditions, with UV irradiation and without sample treatment. Percentage reduction in cytotoxicity was calculated based on the difference in the percentage of UV induced cytotoxicity in cells with and without sample treatment as determined by NRU staining technique.

% UV induced cytotoxicity = [(C1-T1) / C1] × 100
C1 = Absorbance due to cell viability in unexposed sample treated cells.
T1 = Absorbance due to cell viability in UV exposed sample treated cells.
% reduction in cytotoxicity = [(C2-T2) / C2] × 100
C2 = % cytotoxicity in UV exposed cells without sample treatment.
T2 = % cytotoxicity in UV exposed cells with sample treatment.

The culture medium removed during NRU staining was used as samples for determining the Reactive oxygen species content after UV B exposure in sample treated cells as compared to the untreated cells.

Antioxidant potential and UV induced reactive oxygen species (ROS) scavenging activity

200μl of the medium samples collected during the above assay were incubated in a black well microplate with 100μl of 0.002% solution of 2,7-dichlorofluorescein diacetate dye for 1 hour at 37°C. The fluorescence readings which were directly proportional to the fluorescence due to ROS generated were taken at a wavelength Ex/Em 485/520 nm in a FLUOstarOPTIMA microplate reader[5].

% reduction in ROS = [(F1 – F2) / F1] × 100
F1 = Fluorescence reading due to ROS generation in untreated UV exposed cells
F2 = Fluorescence reading due to ROS generation in sample treated UV exposed cells

ORAC value of β-glucogallin

Varying concentrations of β-glucogallin in distilled water were pipetted into each well of a 96 well black microplate, containing 10×10^{-2}M 2,2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH) made in 75mM potassium phosphate buffer (*p*H 7.4) and 4.8×10^{-7}M disodium fluorescein dye. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) a water-soluble derivative of vitamin E was used as a reference compound. Trolox standard from 12.5 – 200μM was also kept under similar conditions. Fluorescence readings were taken in a Fluostar Optima Microplate Reader at 485/520nm after every 1 minute for 35 minutes (f_1……..f_{35}). The final ORAC values were calculated by using a quadratic regression equation ($Y = a + bX + cX^2$) between the Trolox concentration (Y) (μM) and the net area under the Fluorescence decay curve (X) and were expressed as micromoles of Trolox equivalents per gram[6].

Inhibition of melanin generated on UV exposure:

The melanin content was quantitated in mouse melanoma cells by extracting the intracellular melanin with 1N NaOH [7]. B16F1 mouse melanoma cells were

plated in 96 well flat bottomed clear microplate at a seeding density of 5000 cells per well and incubated at 37°C in 95% air and 5% CO_2 in a CO_2 incubator for 24 hours. The cells were then treated with varying concentrations of β-glucogallin in PBS (Phosphorus buffer solution) and exposed to UV B irradiation by three UV B lamps of 33.3 µW cm^{-2} intensity at a distance of 30 cm from the source for 7 minutes. After exposure to UV B irradiation, the sample containing PBS was replaced with fresh medium without sample and incubated in CO_2 incubator. Control cells were also maintained under similar conditions without sample treatment. The UV B irradiation was repeated thrice every 24 hours and at the end of 72 hours, the medium was tapped off, cells were washed once with PBS and the melanin was extracted in 1N NaOH. The absorbance of the melanin was read at 405 nm in a Fluostar Optima microplate reader.

% reduction in melanin = [(M1 – M2) / M1] × 100
M1 = Absorbance due to melanin content in untreated UV exposed cells
M2 = Absorbance due to melanin content in sample treated UV exposed cells

Results

Antioxidant potential, inhibition of UV induced cytotoxicity and ROS generation

The *in vitro* studies showed that β-glucogallin of the fruit extract of Amla had a significant antioxidant potential with an ORAC value of 4200 ± 220 µmol Trolox equivalents/gram and significant inhibitory potential of UV induced cytotoxicity and ROS generation. As given in Table 1, treatment of Swiss 3T3 mouse fibroblast cells with 10 to 80µg/ml of β-glucogallin, reduced the cytotoxicity due to UV exposure by 30 to 90% in comparison to the untreated cells. At the same concentrations it reduced the ROS generation by 42 to 82% in comparison to the untreated cells. Plate 1a & b show decrease in UV induced cytotoxicity in Swiss 3T3 mouse fibroblast cells on treatment with β-glucogallin.

Table 1—Photoprotection by β-glucogallin on exposure of cell cultures to UV B irradiation

β-glucogallin (µg/ml)	*% reduction in cytotoxicity	♦% reduction in ROS generation	‡% reduction in melanogenesis
10	30 ± 2	42 ± 5	15 ± 2
20	52 ± 4	59 ± 2	32 ± 4
40	62 ± 3	72 ± 3	42 ± 3
80	90 ± 2	82 ± 2	55 ± 7

Values are expressed as Mean ± SEM and for each observation n = 12
*% reduction in cytotoxicity with respect to untreated Swiss 3T3 fibroblast cells
♦% reduction in ROS generation with respect to untreated Swiss 3T3 fibroblast cells
‡% reduction in melanogenesis with respect to untreated B16F1 mouse melanoma cells

Inhibition of UV induced melanogenesis

As given in Table 1, treatment of B16F1 mouse melanoma cells with 10 to 80µg/ml of β-glucogallin, reduced the melanogenesis due to UV stress by 15 to 55% in comparison to the untreated cells. Plate 2a & b show decrease in UV induced melanogenesis in B16F1 mouse melanoma cells on treatment with β-glucogallin.

Discussion

UV irradiation is the major environmental factor responsible for high incidence of premature skin ageing, referred to as photo-ageing, as well as skin cancer and melanoma. UV B irradiation has been demonstrated to produce reactive oxygen species

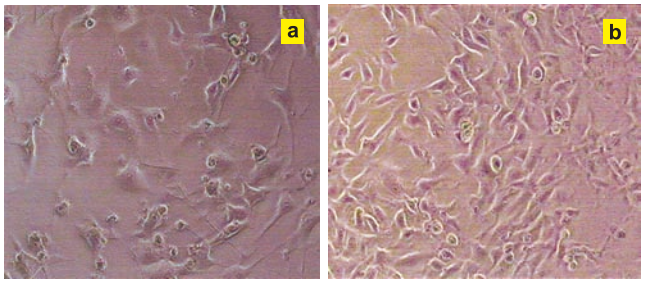

Plate 1—a: Untreated Swiss 3T3 mouse fibroblast cells showing cytotoxicity after UV exposure; b: Swiss 3T3 mouse fibroblast cells treated with 80µg/ml of β glucogallin showing 90% reduction in cytotoxicity after UV exposure

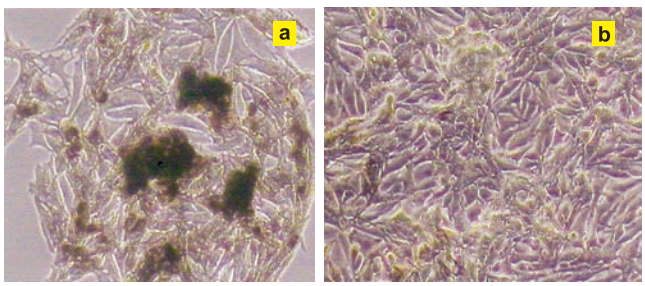

Plate 2—a: Untreated B16F1 mouse melanoma cells showing melanogenesis after UV exposure; b: B16F1 mouse melanoma cells treated with 80µg/ml of β glucogallin showing 55% reduction in melanogenesis after UV exposure

(ROS) in the cells and skin, which induces the synthesis of matrix metalloproteinases (MMPs), causing skin photoaging[8]. Another biological process that is aggravated due to UV and which is of major cosmetic as well as health concern is melanin enhancement resulting in pigmentation and eventually skin tan. The disruption of melanosomal melanin on UV exposure might be an early event in the etiology and progression of melanoma, leading to increased oxidative stress and mutation[9]. Melanin production is induced by UV radiation as a defense mechanism and prolonged UV exposure results in skin tanning. The significant ORAC value and ROS inhibitory potential of β-glucogallin imply that the free radical scavenging mechanisms of β-glucogallin can prevent UV induced adversities like cytotoxicity and enhanced melanogenesis.

Conclusion

β-Glucogallin has a significant role in the photoprotection efficacy of Amla fruit extract. The ROS inhibitory effect of β-glucogallin increases the tolerance of cells to UV B stress, also it inhibits UV induced melanogenesis and prevents skin tanning due to sun exposure. The present work shows the significant photoprotection efficacy of the natural plant fruit extract of *E. officinalis* and emphasizes its potential for sunscreen cosmetic applications.

Acknowledgements

The authors wish to thank the analytical chemistry, phytochemistry and the biological research team of Sami Labs Limited for their facilities and technical assistance.

References

1 Scartezzini P, Antognoni F, Raggi MA, Poli F and Sabbioni C, Vitamin C content and antioxidant activity of the fruit and of the ayurvedic preparation of *Emblica officinalis* Gaertn., *J Ethnopharmacol*, 2006, **104**, 113-118.

2 Pozharitskava ON, Ivanova SA and Shikov AN, Separation and evaluation of free radical scavenging activity of phenol components of *Emblica officinalis* extract by using an HPTLC-DPPH method, *J Sep Sci,* 2007, **30**, 1250-1254.

3 Majeed M, Bhat B, Jadhav AN, Srivastava JS and Nagabhushanam K, Ascorbic Acid and Tannins from *Emblica officinalis* Gaertn. Fruits-A Revisit, *J Agric Food Chem*, 2009, **57**, 220-225.

4 Repetto G, del Peso A and Zurita JL, Neutral red uptake assay for the estimation of cell viability/cytotoxicity, *Nat Protoc,* 2008, **3** (7), 1125-1131.

5 Bergman M, Perelman A, Dubinsky Z and Grossman S, Scavenging of reactive oxygen species by a novel glucurinated flavonoid antioxidant isolated and purified from spinach, *Phytochemistry*, 2003, **62**, 753-762.

6 Ou B, Hampsch-Woodill M and Prior RL, Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe, *J Agric Food Chem*, 2001, **49**, 4619-4626.

7 Mas-Chamberlin C, Peschard O, Leroux R, Mondon Ph, Lamy F *et al*, Di-acetyl-nor-aporphines: Novel Molecules and Novel Mechanism to inhibit Melanogenesis, *SOFW-Journal*, 2004, **130**, 2-10.

8 Ho JN, Lee YH, Park JS, Jun WJ, Kim HK *et al*, Protective effects of aucubin isolated from *Eucommia ulmoides* against UVB-induced oxidative stress in human skin fibroblasts, *Biol Pharm Bull,* 2005, **28** (7)**,** 1244-1248.

9 Gidanian S, Mentelle M, Meyskens FL Jr and Farmer PJ, Melanosomal damage in normal human melanocytes induced by UVB and metal uptake-a basis for the pro-oxidant state of melanoma, *Photochem Photobiol*, 2008, **84** (3), 556-564.