Experimental Gerontology 111 (2018) 94–106

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Age-induced diminution of free radicals by Boeravinone B in *Caenorhabditis elegans*

Laxmi Rathor, Rakesh Pandey*

Aging Biology Lab, Microbial Technology and Nematology Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226 015, India
Academy of Scientific & Innovative Research (AcSIR), New Delhi, India

ARTICLE INFO

Section Editor: Christiaan Leeuwenburgh

Keywords:
Boeravinone B
Stress resistance
Age-related disorders
Longevity
Caenorhabditis elegans

ABSTRACT

Oxidative damage is accrual of molecular deterioration from reactive oxygen species (ROS) while decrease in generation of ROS is related with free radical scavenging enzymes. *Boerhaavia diffusa L.* (Nyctaginaceae) derived novel molecule Boeravinone B (BOB) possesses a variety of pharmacological activities, yet their anti-aging potential has not been explored. The aim of the present study was to elucidate the mechanism of BOB mediated oxidative stress resistance and lifespan extension in *Caenorhabditis elegans*. The results showed that the BOB significantly extends the lifespan of *C. elegans* with its anti-oxidative potential via reducing accumulation of reactive oxygen species (ROS). BOB was found to recover the shortened lifespan of oxidative stress prone mutants *mev-1* and *gas-1* (14.75 and 16.11%, respectively). Additionally, this finding supported by the reduced ROS levels seen in BOB treated worms. Further, the effective concentration of BOB (25 μM) significantly enhanced the expressions of target genes such as superoxide dismutase (SOD-3), glutathione-S-transferase (GST-4) and heat shock protein (HSP-16.2) fused to green fluorescent protein (GFP), and it does so by modulating the stress-related signaling pathways (SEK-1) and transcription factors (SKN-1/Nrf and DAF-16/Foxo). Moreover, BOB exposure (25 μM) caused significant changes of age-dependent biomarkers such as pharyngeal pumping, body bend, locomotor activity and lipofuscin accumulation were also showed that BOB retards the aging. Overall, the findings highlight the antioxidant supplement triggering pharmaceutical potential of BOB which may serve as a new future perspective for healthy aging or delayed onset of oxidative related diseases.

1. Introduction

Reactive oxygen species (ROS) are generated during aerobic respiration and several metabolic reactions. Overproduction of ROS causes severe damage to the variety of proteins, lipids and DNA, ultimately contributing aging and age-related disorders. It is well established that the large number of flavonoids as a dietary supplements have shown to induce phase II detoxification genes viz., glutathione-*S*-transferases (GSTs), glutathione peroxidase (GP_X) and glutathione reductase (GR) through activating Nrf2 (NF-E2-related factor) signaling. The induction of these enzymes protects cells from the damage associated to oxidative stress in diverse in vivo experimental conditions (Son et al., 2008; Lee et al., 2003; Wang et al., 2013). Therefore, the addition of phytomolecules through diet could help to prevent pathogenesis of age-related diseases and contribute to delay aging, or in other words extend health span of organisms.

The nematode *Caenorhabditis elegans* is an important model organism to study aging and age-related diseases as the major signaling pathways that regulate longevity and stress resistance in mammals are well conserved in *C. elegans* (González et al., 2009). *C. elegans* has been increasingly proven to be a good model organism for the discovery of novel phytomolecules that modulate the aging processes (Zheng et al., 2016; Negi et al., 2016; Pant and Pandey, 2015). Numerous studies have successfully identified several natural antioxidants such as resveratrol (Kan et al., 2010), epigallocatechin gallate (Abbas and Wink, 2009), quercetin (Kampkötter et al., 2008) and others that play a promising role in aging. Aging is an inevitable biological process and excessive production of reactive oxygen species (ROS) generates oxidative stress, a main risk factor for several age-related disorders. Production of free radicals is an inescapable process in the course of cellular metabolism (Getoff, 2007) and there is well known increasing evidence that accumulation of free radicals leads to cellular damage (Pole et al., 2016; Gladyshev, 2014). Antioxidants alleviate this damage by scavenging the ROS production and thereby promoting longevity and preventing age-related disorders. Thus, for healthy lifespan and to relieve this stress, an urgent need is to identify some novel herbal remedies with least toxicity

* Corresponding author.
E-mail address: r.pandey@cimap.res.in (R. Pandey).

https://doi.org/10.1016/j.exger.2018.07.005
Received 12 July 2017; Received in revised form 27 June 2018; Accepted 3 July 2018
Available online 10 July 2018
0531-5565/

effects. In this context, medicinal plants provide a high diversity of natural products, which can be exploited as potential antiaging agents.

Boerhaavia diffusa (Nyctaginaceae) is an important medicinal plant and is widely used in traditional system of medicine worldwide (Getoff, 2007). In pharmacological studies, the immunomodulatory effects (Mehrotra et al., 2002), antidiabetic activity (Pari and Satheesh, 2004), analgesic (Goyal et al., 2010), antibacterial (Awasthi and Verma, 2006) properties have been attributed to its roots. In this study, we evaluated the health-promoting anti-aging and anti-stress potentials of Boeravinone B (BOB) phytomolecule, a major retinoid isolated from the roots of *Boerhaavia diffusa*, using *C. elegans*.

2. Materials and methods

2.1. Test compound and toxicity assays

BOB (procured from Natural Remedies, India) was dissolved in 10% DMSO to prepare stock solutions. The test concentrations were prepared with different doses of BOB (5, 25, 50, 100, 200, 500 µM and 1 mM) whereas 0.1% DMSO served as a control. For toxicity assays, the selected concentrations were spotted directly onto a 35 mm or 60 mm Petri dish containing 2 mililiter/5 mililiter of NGM. Percentage survival was observed after 24 h. The experiment was repeated in three independent trials.

2.2. Strains and culture conditions

Nematodes were maintained as previously described (Brenner, 1974). The following strains were used in this study: N2: (Bristol, wild type), TK22: *mev-1*(*kn1*), MQ887: *isp-1*(*qm150*), CW152: *gas-1*(*fc21*), CB1370: *daf-2* (*e1370*), GR1307: *age-1*(*hx546*), GR1307: *daf-16*(*mgDf50*), EU1: *skn-1*(*zu67*), KU4: *sek-1*(*km4*), CL2166: *dvIs19* (*gst-4p::gfp*), CF1553: *muIs84* (sod-3p::*gfp* + rol-6), CL2392: *hsp-16.2*(*dvIs47*), TJ356: *daf-16* (*zIs356*).

2.3. Lifespan analysis assay

C. elegans lifespan assay was propagated at 20 °C on triplicate NGM plates (50 worms/plate). Synchronization of worm culture was achieved by treating gravid hermaphrodites with bleach (0.5 mililiter 5% sodium hypochlorite; 4.5 mililiter 0.5 M sodium hydroxide) and recovering the hatched L1 larvae on NGM/OP50 plates. 50 worms from L1 stage were transferred to NGM plates spotted with *E. coli* OP50 as a food source as previously described (Rathor et al., 2015; Onken and Driscoll, 2010) and containing 5 µM, 25 µM and 50 µM BOB concentrations. 50 µM 5-Fluoro- 2'deoxyuridine (FUdR) was likewise appended on to the NGM plates to inhibit the progeny growth and transferred every 3–4 days to new OP50 seeded NGM plates. All the experiment treatments started from the L1 stage unless otherwise stated. Surviving and dead animals were counted every other day until all individuals had died. A total of three independent trials were performed per treatment. To test the antibacterial effect of BOB, antimicrobial activity with effective dose of BOB(25 µM) was performed as described (Suryanto et al., 2012). Three independent trials were performed for all the treatments and the data shown represents the mean lifespan per trial.

2.4. Progeny profiles

The assay was performed according to a previously reported method (Rathor et al., 2016). As described in lifespan assay, wild type N2 worms were allowed to lay eggs started from day l (10 individuals per plate). Parent BOB treated worms were transferred to new plates every day during the reproduction day, and the plates with eggs were left at 20 °C for 24 h to allow the eggs to hatch. The number of progeny was allowed to develop for 48 h for quantification of hatching nematodes.

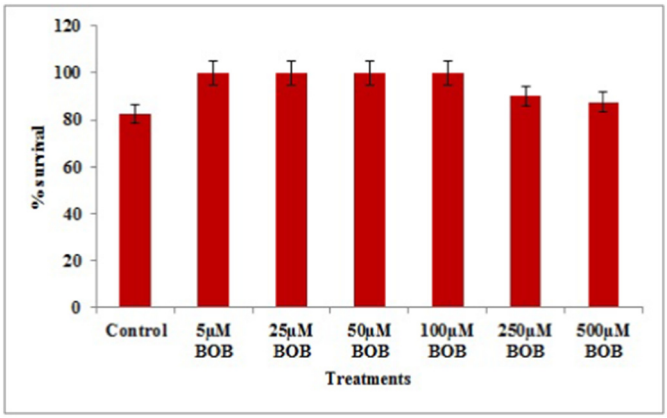

Fig. 1. Toxicity assay done at 20 °C with wild-type N2 worms. Worms were grown on the NGM agar plate at 20 °C in the absence or presence of different doses of BOB (5 µM to 500 µM). The results were evaluated after 24 h.

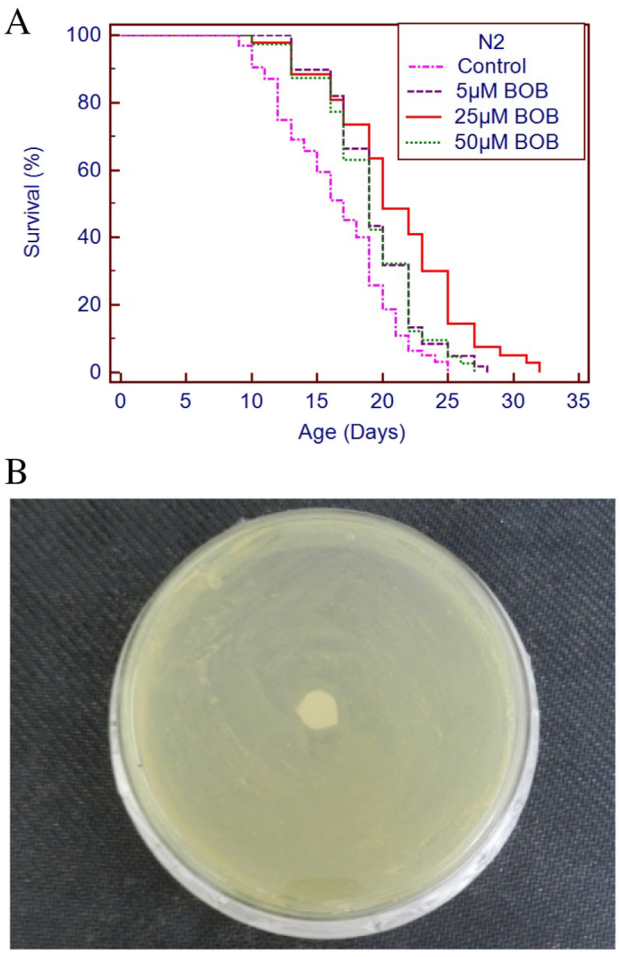

Fig. 2. (A) Treatment with BOB prolonged lifespan of *C. elegans* (worms were grown on BOB treated NGM plates at different concentrations viz., 5 µM, 25 µM, 50 µM). (B) Antimicrobial assessment of BOB. Effect of BOB on *E. coli* proliferation. Bacterial growth test conducted with pure OP50, or OP50 with 25 µM of BOB. No zone of inhibition was seen after BOB treatment.

This procedure was continued until the parental worms were dead or stopped producing progeny. Assay for each experiment was performed in triplicate.