Free radical scavenging activity biology essay

Science, Biology

To measure the antioxidant activity of EPs7630® the DPPH˚ assay was performed. In this experiment 0. 2 mM DPPH˚ solution was added to different concentrations of EPs7630® and the free radicals were neutralized through hydrogen donation. EPs7630® scavenges free radicals in a dose dependant mode (Fig 3. 1) with an IC50 (the concentration of sample that causes 50% loss of DPPH o activity; the lower IC50, the higher antioxidant activity) of 14. 7 ± 0. 85 µg/ml. EGCG (epigallocatechin gallate) is a main polyphenol in green tea with antioxidative properties. It was used as a positive control with an IC50 of 4. 24 ± 0. 32 µg/ml. In conclusion, EPs7630® shows a good free radical inhibitory effect in vitro (Fig. 3. 1). Fig 3. 1 DPPH˚ free radical scavenging activity of EPs7630® and EGCG. Results were obtained from three independent experiments. Error bars stand for means ± SD.

3. 2 Superoxide anion radical scavenging activity

In a non enzymatic system, consisting of PMS-NADH and NBT, superoxide anions were generated. After adding different concentrations of EPs7630® to the mixture, the absorption was measured at 560 nm, indicating the consumption of superoxide anion radicals in the mixture. EGCG served as a positive control (Abbas and Wink, 2009). Superoxide generation was inhibited up to 60% and 54% by 30 µg/ml of EPs7630® and EGCG respectively. EPs7630® inhibits superoxide anion generation in a dose dependant way with an IC50 of 24. 3 ± 0. 3 µg/ml while EGCG shows an IC50 of 17. 15 ± 0. 74 µg/ml. Fig 3. 2 Superoxide anion radical scavenging activity of EPs7630® and EGCG. Results were obtained from three independent experiments. Error bars stand for means ± SD.

3. 3 EPs7630® increases stress resistance in C. elegans

To investigate the antioxidant properties of EPs7630® in vivo, transgenic worms (strain TJ375) which do not show any GFP expression under normal conditions were used (Rea et al. 2005). After adding juglone, which induces intracellular superoxide radicals, an excessive GFP expression in the pharynx of the transgenic hsp-16. 2:: GFP (gpls1) nematodes is noticeable (Fig 3. 3a). Comparing to control the expression of hsp-16. 2:: GFP was decreased by 31. 29% and 46. 77% respectively. In nematodes which were pretreated with 40 µg/ml and 50 µg/ml EPs7630® for 48 h before adding juglone (Fig 3. 3b, c). L1 stage worms were treated in Petri dishes containing S-medium as a standard medium in control (a), EPs7630® 40 µg/ml solved in water in S-medium (b), EPs7630® 50 µg/ml solved in water in S-medium (c), respectively, for 48 h. Transferring the worms to new media containing 20 µM juglone for 24 h caused GFP expression in their pharynx. Analyzing at least 25 worms in each group with Image J software showed a reduction in GFP mean pixel density in pretreated worms with 40 µg/ml and 50 µg/ml EPs7630®. In conclusion, the mean pixel densities in pretreated worms with 40 µg/ml (1869 ± 93. 63) and 50 µg/ml EPs7630® (1448 ± 113. 42) were reduced compared to control (2720 ± 31. 82).(a) C: UsersLEILADesktopcontrol. jpg (b)C: UsersLEILADesktopEPs7630 50. jpg(c)C: UsersLEILADesktopEPs7630 50. jpgFig 3. 3 Effects of EPs7630® on GFP expression in TJ373 hsp-16. 2:: GFP (gpls1) strain. Data were obtained from three independent experiments. Error bars stand for means ± SD. $P < 0.0001$. Pretreatment of C. elegans with 40 (b) and 50 µg/ml (c) concentration

of EPs7630® for 48 h reduced GFP mean pixel density compared to control (a).

3. 4 EPs7630® reduces the intracellular ROS level in C. elegans

In this experiment a cell permeating reagent 2', 7' –dichlorofluorescein diacetate (DCFDA), which is a fluorogenic dye, was used. DCFDA diffuses into the cell and is capable of measuring hydroxyl, peroxyl and other reactive oxygen species (ROS) activity within the cell. DCFDA is deacetylated by cellular esterases to a non-fluorescent compound, which is later oxidized by ROS into 2', 7' –dichlorofluorescin (DCF). DCF is a highly fluorescent compound which can be detected by fluorescence spectroscopy with maximum excitation and emission spectra of 495 nm and 529 nm respectively. Treatment of N2 wild type C. elegans with EPs7630® for 72 h resulted in a noticeable decrease regarding intracellular ROS levels. Adding 40 and 50 µg/ml of EPs7630® to the medium caused intracellular ROS reduction by 22. 05% ($p < 0. 01$) as well as 39. 4% ($p < 0. 01$), respectively (Fig 3. 4). Our results suggest that EPs7630® is capable of reducing the intracellular ROS levels in vivo. Fig 3. 4 Reduction of intracellular ROS levels in wild type N2 C. elegans by EPs7630® treatment for 72h 60 worms were collected and analyzed for samples and control. Values are means ± SE from three independent experiments and the total number of worms was 540 ($p < 0. 01$).

3. 5 EPs7630® increases survival rate in C. elegans

To evaluate the survival rate of wild type N2 C. elegans, age synchronized L1 larvae were treated with 40 and 50 µg/ml of EPs7630® on the day after

hatching for 48 h. In order to measure the survival rate, a lethal dose of juglone (300 µM), causing oxidative stress, was added to the medium. Fig. 3. 5 indicates that the N2 wild type worms pretreated with 40 and 50 µg/ml of EPs7630® were able to survive better under lethal oxidative stress than untreated (control) worms. After causing stress with juglone, 22. 33 ± 1. 45 % and 25. 33 ± 1. 45 % of the worms which were treated with 40 and 50 µg/ml of EPs7630® for 48 h were alive respectively. This shows that the survival rate was increased by 10%, $p < 0.01$ and 13%, $p < 0.001$ in the treated worms comparing to control. Fig. 3. 5 Increase of survival rate after oxidative stress. Pretreatment of N2 wild type C. elegans with EPs7630® (40 and 50 µg/ml for 48 h) increased the survival rate after oxidative stress caused by adding lethal dose of juglone (300 µm) for 24 h. The percentage of mean was calculated from three independent trials, consisting of 300 worms in each one. Error bars stand for means ± SE.

3. 6 EPs7630® increases life span in C. elegans

To investigate the anti-aging effect of EPs7630® synchronized adult hermaphrodite worms were treated with 50 µg/ml of EPs7630® in three independent experiments consisting of 50 worms for each group. Except for BA17-(fem-1) strain, which is temperature sensitive and does not show progeny in 25 ˚C, other strains were kept in 20 ˚C. Fig 4. 5A indicates an 31. 52% increase of life span in wild type N2 worms after treatment with 50 µg/ml of EPs7630® (control: 10 ± 0. 5 n; EPs7630®: 14 ± 0. 9 n; P value = 0. 0003, Table 3. 1). Fig. 4. 5B shows that treatment of BA17-(fem-1) strain with 50 µg/ml of EPs7630® increased life span by 26. 61% (control: 9 ± 0. 4 n; EPs7630®: 12 ± 0. 6 n; P value = 0. 0002, Table 3. 1). As discussed

before this BA17-(fem-1) strain is valuable in life span assays because of its temperature sensitivity, which avoids progeny formation with the help of temperature increase to 25 ˚C. Therefore worms can be transferred to the new medium every other day instead of daily. Furthermore to investigate the mode of action of EPs7630®, mev-1 (TK22 Kn1) strain was used. In this strain, free radicals are overproduced which results in an acceleration of aging process (Ishii et al, 1998). This strain was treated with 50 µg/ml of EPs7630®, which increased the mean life span by 21. 8% (control: 10 ± 0. 5 n; EPs7630®: 12 ± 0. 6 n; P value = 0. 0102, Fig. 4. 5C).

Table 3. 1 Effects of EPs7630® on life span in C. elegans

Strain

Treatment (µg/ml)

Total

Of worms

Mean ± SE

(day)

Extension

Maximum

life span

P value

Wild type N2

Control5010 ± 0. 5

-

21

-

EPs7630®(50 µg/ml)5014 ± 0. 931%260. 0003

BA17-(fem-1)

Control509 ± 0. 4

-

18

-

EPs7630®(50 µg/ml)5012 ± 626%230. 0002

mev-1 (TK22 Kn1)

Control5010 ± 0. 5

-

20EPs7630®(50 µg/ml)5012 ± 0. 621%240. 0102Fig 4. 5 Kaplan Meyer presentation of life span after treatment with EPs7630®C: UsersLEILADesktopLeila nemudar3 copy. jpgAC: UsersLEILADesktopLeila nemudar 2 copy. jpgBC: UsersLEILADesktopLeila nemudar 1 copy. jpgC

3. 7 EPs7630® prolong longevity of C. elegans by activating the FOXO transcription factor

Life span increasing effect of EPs7630®. was investigated. Fig 4. 5 indicates that treating wild type N2 worms with 50 µg/ml of EPs7630® markedly increased their life span by 31% as compared to untreated worms. Moreover,

EPs7630® increased C. elegans resistance against oxidative stress. To study the molecular mode of EPs7630®, on longevity in C. elegans, the TJ356 (DAF-16:: GFP) strain was employed. In this strain DAF-16:: GFP fusion protein is constructed as reporter to reveal the subcellular distribution of DAF-16 (Henderson and Johnson, 2001) Several well-known biological pathways relevant for life span and stress resistance are regulated through DAF-16 transcription factor (Line et al, 1997; Patridge and Gems, 2002; Lee et al, 2003). Presence of DAF-16 in the nucleus is required for activating transcription of various genes which increase stress resistance against environmental stresses (e. g heat, H2O2, radiation, etc) as well as longevity. Localization of DAF-16:: GFP in the nucleus occurs as a result of either heat shock treatment, mutational inactivation of DAF-2 or oxidative stress with pro-oxidants such as juglone (Henderson and Johnson, 2001). Our results demonstrate that treating the transgenic mutant TJ356 with 50 µg/ml of EPs7630® caused the translocation of DAF-16 from the cytosol to the nucleus. (Fig 5. 5D) indicating that EPs7630® activates DAF-16 transcription factor through insulin/IGF-1 signaling pathway which is essential for the life span extension in treated worms.

Fig 5. 5 Effects of various treatments on DAF-16:: GFP localization in transgenic C. elegans (TJ356).

(A) Negative control; (B) Heat shock (37˚ C for 15 min) and (C) oxidative stress (20 µM juglone, 1 h) as positive controls; (D) Treatment with 50 µg/ml EPs7630®. for 1 h. All data were obtained from three independent experiments each consist of 100 worms. C: UsersLEILADesktopupleur margin pos und neg. jpg(A)C: UsersLEILADesktoppos contr 37. jpg(B)C:

UsersLEILADesktoppos contr egcg. jpg(C)C: UsersLEILADesktoppos contr 37. jpg(D)

3. 8 Discussion

The antioxidative properties of natural compounds or plant extractsresult from metal chelating, free radical scavenging (hydrogendonating capability or free radical quenching), or allthese properties together. The DPPH● assay is a valid and easymethod for evaluating antioxidant activity. EGCG revealed astrong dose-dependent DPPH· radical scavenging activity (whichwas greater than that of L-ascorbic acid at the same molar concentration)because it contains eight phenolic OH groups [18]; this agrees with an earlier finding that EGCG has a strong DPPHscavenging activity [19]. Superoxide anion radical scavengingalso is biologically important, because it contributes to the formationof ROS, which induce oxidative damage in lipids, proteins, and DNA. It has been reported that superoxide anion radicalsplay an important role in the aging process [20]. ●_ Fig. 2 illustratesthe dose-dependent scavenging activity of EGCGagainst superoxide anion radicals, which may be due to the reductionpower of its phenolic hydroxy groups. The obtained resultsfrom DPPH● and superoxide anion assays suggest thatEGCG has strong free radical scavenging activity in vitro. Apparently, EGCG is also active in vivo, as it significantly attenuatesthe intracellular level of H2O2 in C. elegans (●_ Fig. 3). This resultTo evaluate the antioxidant properties of our compounds DPPH assay was conducted. EPs7630® showed a good dose-dependent free radical scavenging activity compared to EGCG as a positive control, resulting from the existence of polyphenolic compounds as well as 7-hydroxy derivatives coumarins in its structure. To examine the effect of

polyphenols on the longevity as well as complicated phenotype of aging, the model organism C. elegans was applied. Polyphenols are a strong substance group, extending the life span in C. elegans (Wu et al, 2002; Ishii et al, 2004; Wilson et al, 2006; Abbas and Wink, 2009; Pietsch et al, 2009; Saul et al, 2009; Wei et al, in press). In this study, we investigated whether polyphenols of tannic acid as well as EPs7630® have the ability to delay aging and prolong life span in C. elegans. The correlation between these protective actions of polyphenols to their antioxidatant activity was also studied. Metal chelating, free radical scavenging and hydrogen donating alone or altogether are main mechanisms causing antioxidant features of natural compound as well as plant extract. Tannic acid and EPs7630® showed antioxidant activity in vitro using L-ascorbic acid and EGCG as positive controls (Abbas and Wink, 2009) (fig ?????). Treatment with EPs7630® (50 µg/ml) extended the life span of the wild type N2 as well as BA-17 [fem-1 (hc17)] worms. To further study the preventing effect of EPs7630® as an antioxidant against oxidative stress (in vivo), 50 µg/ml concentration of EPs7630® was applied to mev-1(kn1) mutants. The loss-of-function mutation in these worms leads to an excessive oxidative stress followed by a shorter life span compared to wild type N2 worms (Senoo-Matsuda et al, 2001). In the present study, EPs7630® (50 µg/ml) exposure increased the longevity of mev-1(kn1) mutants (Fig and Table ??????) confirmed that the antioxidant ability of EPs7630® is the trigger for longevity. Interestingly these results are consistent with the EGCG study in C. elegans (Abbas and Wink, 2009) demonstrated that mev-1(kn1) mutants benefit from the treatment with 55 µM concentration of EGCG. also showed life span extension in mev-1(kn1) mutants which have a shorter life

span compared to wild type N2 worms as a result of excessive oxidative stress (Senoo-Matsuda et al, 2001). the worms with Tannic acid did not extend the life span in the mev-1(kn1) mutantsTannic acid shows antimicrobial activity against E. coli (Chung et al, 1998; Kim et al, 2004). C. elegans utilizes E. coli as a food source. Therefore, It could be argued that