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Fisetin Protects DNA Against Oxidative Damage and Its Possible Mechanism

Suppl. 1. Detailed experimental protocols

Protective effect against hydroxyl-induced DNA damage (DNA degradation assay)

The protective effect against •OH-iduced DNA damage was estimated using the method developed by our laboratory.[S1] Briefly, the sample methanol solutions were separately aliquoted into mini tubes. After completely evaporating methanol solvent in the each tube at 60°C, the sample residue was added by 300 μL phosphate buffer (0.2 M, pH 7.4), followed by 50 μL DNA sodium (10 mg/mL), 75 μL H_2O_2 (33.6 mM), 50 μL $FeCl_3$ (3.2 mM), 100 μL Na_2EDTA (0.5 mM), and 75 μL ascorbic acid (12 mM). The total volume of the reaction mixture was brought to 650 μL with buffer. After incubation at 55°C for 20 min, 250 μL of trichloroacetic acid (10%, w/w) was put in the tube. The color of mixture was obtained after heating at 105°C for 15 min with 150 μL 2-thiobarbituric acid (TBA, 5% in 1.25% NaOH aqueous solution), and was measured at 530 nm (Jinhua 754-PC, Shanghai, China) against the buffer (as the blank). The protective percentage is expressed as follows:

$$\text{Inhibition\%} = \frac{A_0 - A}{A_0} \times 100 \qquad \text{(Equation 1)}$$

Where A_0 indicates the absorbance of blank and A indicates the absorbance of tested sample.

Scavenging ability on •OH radical (deoxyribose degradation assay)

The •OH radical-scavenging assay was performed in accordance with deoxyribose degradation method improved by our laboratory.[S2] In brief, sample was dissolved in ethanol at appropriate concentrations. Various amounts of sample ethanolic solutions were then separately taken into mini tubes. After evaporating the sample solutions in tubes to dryness, the sample residue was then added by 50 μL deoxyribose (50 mM), 50 μL Na_2EDTA (1 mM), 50 μL $FeCl_3$ (3.2 mM), 50 μL H_2O_2 (50 mM), and 50 μL ascorbic acid (1.8 mM). The total volume of the reaction mixture was adjusted to 800 μL with buffer and mixed thoroughly. After incubation at 50°C for 20 min, the reaction was terminated by 50 μL trichloroacetic acid (10%, w/w). The color was then developed by addition of 30 μL 2-thiobarbituric acid (5%, in 1.25% NaOH aqueous solution) and heated in an oven at 115 °C for 15 min. The mixture was cooled and absorbance was measured at 530 nm in a spectrophotometer (Jinhua 754 PC, Shanghai, China) against the buffer (as blank). The •OH radical scavenging activity was expressed as Equation 1.

Scavenging ability on $•O_2^-$ radical (pyrogallol autoxidation assay)

The superoxide anion ($•O_2^-$) scavenging activity was determined by the method stablished by our laboratory.[S3] Briefly, 30-150 μL sample solution (1 mg/mL) was brought to Tris-HCl buffer (0.05M, pH 7.4) containing Na_2EDTA (1 mM) and the total volume was 980 μL. Then about 20 μL pyrogallol solution (60 mM, in 1 mM HCl) was poured into the 980 μL mixture. After vigorous mixing, it was read at 325 nm every 30 s for 5 min. The $•O_2^-$ scavenging ability was calculated as:

$$\text{Inhibition\%} = \frac{\Delta A_{325nm,control} - \Delta A_{325nm,sample}}{\Delta A_{325nm,control}} \qquad \text{(Equation 2)}$$

Here, $\Delta A_{325nm,\ control}$ is the increase in A_{325nm} of the mixture without the sample and $\Delta A_{325nm,\ sample}$ is that with the sample.

DPPH• radical scavenging assay

Scavenging activity on DPPH• radicals was assessed according to the method reported by Li.[S4] Briefly, 500 μL of the sample methanolic solution (at least 5 different concentrations were prepared) was mixed with 1000 μL DPPH• solution (100 μM in methanol, prepared daily). The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 519 nm against a blank. The DPPH•-scavenging activity of each solution was calculated as percent inhibition, according to the Equation 1**.**

$ABTS•^+$ radical scavenging assay

$ABTS•^+$ scavenging activity was evaluated by the method.[S5] The $ABTS•^+$ was produced by mixing 200 μL ABTS diammonium salt (7.4 mM) with 200 μL $K_2S_2O_8$ (2.6 mM). After incubation in the dark for 12 h, the mixture was diluted with methanol (about 1:50) so that its absorbance at 734 nm was 0.70±0.02. Then, the diluted $ABTS^+•$ solution (800 μL) was brought to 200 μL salvianolic acid B ethanolic solution at various concentrations, thoroughly mixed. After reaction mixture stood for 6 min, the absorbance at 734 nm was read on a spectrophotometer. The percentage inhibition was calculated also by Equation 1.

Cupric ions (Cu^{2+}) reducing power assay

Cu^{2+}-reducing power assay was carried out according to the method.[S6] To 125 μL $CuSO_4$ aqueous solution (10 mM), 125 μL neocuproine solution (7.5 mM in CH_3OH) and salvianolic acid B solutions at appropriate concentrations were added. Then, the total volume was adjusted to 1000 μL with CH_3COONH_4 buffer and mixed vigorously. Absorbance against a buffer blank was measured at 450 nm after 30 min. The relative Cu^{2+}-reducing power was calculated using the formula:

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$$\text{Relative reducing power\%} = \frac{A - A_{min}}{A_{max} - A_{min}} \times 100 \quad \text{(Equation 3)}$$

Here, A_{max} is the maximum absorbance in the test and A_{min} is the minimum absorbance in the test. A is the absorbance of tested sample.

Statistical Analysis

All experiments were performed in triplicate, and the data were recorded as the mean ± SD. The IC_{50} value was defined as the final concentration of 50% radical inhibition percentage (or chelating percentage, reducing power). All IC_{50} values were calculated by linear regression and analyzed using Origin 6.0 professional software (Origin Lab Corporation, Northampton, MA, USA). Determination of significant differences was performed using the *T*-test ($p < 0.05$). The analysis was performed using SPSS software (v.12, SPSS, USA).

References

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