Soy Isoflavone Glycitein Protects Against Beta Amyloid-Induced Toxicity and Oxidative Stress in Transgenic *Caenorhabditis elegans*

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Soy isoflavone glycitein protects against beta amyloid-induced toxicity and oxidative stress in transgenic Caenorhabditis elegans

Astrid Gutierrez-Zepeda¹, Ross Santell², Zhixin Wu¹, Marishka Brown¹, YanJue Wu¹, Ikhlas Khan³, Christopher D Link⁴, Baolu Zhao⁵ and Yuan Luo*¹,⁶

Abstract

Background: Epidemiological studies have associated estrogen replacement therapy with a lower risk of developing Alzheimer’s disease, but a higher risk of developing breast cancer and certain cardiovascular disorders. The neuroprotective effect of estrogen prompted us to determine potential therapeutic impact of soy-derived estrogenic compounds. Transgenic C. elegans, that express human beta amyloid (Aβ), were fed with soy derived isoflavones genistein, daidzein and glycitein (100 µg/ml) and then examined for Aβ-induced paralysis and the levels of reactive oxygen species.

Results: Among the three compounds tested, only glycitein alleviated Aβ expression-induced paralysis in the transgenic C. elegans. This activity of glycitein correlated with a reduced level of hydrogen peroxide in the transgenic C. elegans. In vitro scavenging effects of glycitein on three types of reactive oxygen species confirmed its antioxidant properties. Furthermore, the transgenic C. elegans fed with glycitein exhibited reduced formation of β amyloid.

Conclusion: These findings suggest that a specific soy isoflavone glycitein may suppress Aβ toxicity through combined antioxidative activity and inhibition of Aβ deposition, thus may have therapeutic potential for prevention of Aβ associated neurodegenerative disorders.

Background

Estrogen, a natural steroid long associated with effects on the female reproductive system, also plays a role in the central nervous system (CNS) through binding estrogen receptors located in the brain [1,2]. It has been demonstrated that estrogen has neuroprotective and neurotrophic properties [1-9]. Epidemiological studies suggest that post-menopausal women using Estrogen Replacement Therapy (ERT) have a decreased risk of developing dementia [10-12]. However, the beneficial effect of ERT
on dementia associated with Alzheimer’s disease (AD) is yet inconclusive [13-15]. Although ERT alleviates the symptoms associated with menopause and has positive effects on bones, ERT in post-menopausal women has been linked to a higher incidence of uterine and breast cancer. Consequently, the Selective Estrogen Receptor Modulators (SERMs) compounds that exert tissue specific estrogenic effects may provide the benefits of ERT without the risks. A group of natural SERMs are the soy-derived phytoestrogens, which are structurally similar to estrogen [16], and may serve as an alternative to ERT [17-19].

Soybeans contain a large amount of isoflavones, including genistein (4', 57-trihydroxyisoflavone), daidzein (4', 7-dihydroxyisoflavone), glycine (6-methoxydaidzein) and their glycosides [20]. Experimental evidence suggests that soy isoflavones possess many properties including estrogenic [16], antioxidant [21] hypcholesteronemic [22], and inhibition of cell proliferation and DNA synthesis [23,24]. Phytoestrogens exert estrogen agonist and antagonist characteristics [17], in part because of differential binding affinities for the estrogen receptor (ER) isoforms; with higher affinity for ERβ than for ERα. Areas of the brain responsible for cognitive function and susceptible to AD (basal forebrain, hippocampus, cerebral cortex), express higher levels of ERβ compared to ERα [25]. Thus, interest in these compounds has grown because they could be used as SERMs, to delay or prevent the cognitive decline associated with AD [3,26] without increasing the risk of developing cancer [27].

AD is widely recognized as a serious public health problem [28]. The clinical symptoms of AD begin with memory impairment that eventually progresses to dementia, a process postulated to be the consequence of selective degeneration of nerve cells in those brain regions critical for memory, cognitive performance and personality [29]. AD is characterized by the presence of amyloid beta peptide (Aβ1–42) aggregation and increased oxidative stress, both causing neuronal injury and death [30]. An "amyloid cascade" hypothesis states that accumulation of Aβ deposition initiates a series of downstream neurotoxic events, which result in neuronal dysfunction and death [31]. The strongest evidence supporting this hypothesis comes from molecular genetic studies. Patients with Down’s Syndrome, a disease related to an extra copy of chromosome 21 containing the APP gene, develop AD with the formation of Aβ deposits, an early sign of brain lesion [32]. All familiar forms of AD (FAD)-linked mutations, in the APP gene or two presenilin genes (PS1 and PS2), result in increased production of Aβ42, which is the more amyloidogenic form [33]. Transgenic mice overexpressing the mutant APP develop Aβ-containing amyloid plaques similar to those found in AD. Furthermore, inducing toxicity and cognitive dysfunction by introduc-

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We define PT$_{50}$ as time duration at which 50% worms were paralyzed from 30 hrs after up shift of temperature to 23°C. Statistically, a significant delay of Aβ-induced paralysis was only observed in the worms fed with glycitein (Fig. 1C, Control, PT$_{50}$ = 2.6 ± 0.08 h vs. Glycitein, PT$_{50}$ = 3.3 ± 0.25 h, p = 0.036; Daidzein, PT$_{50}$ = 2.5 ± 0.10 h, p = 0.46; Genistein, PT$_{50}$ = 2.6 ± 0.15 h, p = 0.76; n = 3 assays each drug, 40 worms in each assay group). Although Daidzein accelerated paralysis at the end point, PT$_{50}$ did not indicate significant difference (Fig 1C) compared with that of the controls. It is known that the effective concentration for genistein to activate the estrogen receptor and tyrosine kinases is much lower (nM). Differential concentration effects of genistein might contribute to protection against Aβ toxicity/paralysis. Thus, we conducted experiments using genistein at two lower doses (10 µg/ml and 0.1 µg/ml). Aβ-induced paralysis was not affected in the worms fed with either of the two concentrations (data not shown), supporting the view that the effect of glycitein is specific.

To determine the overall effect of the isoflavones on the behavioral of the C. elegans, we conducted oxidative stress sensitivity assay and life span assay. We found that the C. elegans fed with glycitein were more resistant toward an oxidative stressor Juglone than the worms fed with daidzein and genistein (data not shown). However, the maximum life span was not affected in the C. elegans CL2006 fed with glycitein compared with untreated control worms (data not shown).

2. Glycitein attenuates levels of H$_2$O$_2$ in the Aβ-expressing C. elegans & in vitro

Given that soy isoflavones are potent antioxidants, we determined whether the antioxidative properties of the isoflavones might contribute to protection against Aβ-toxicity. Previously, we established an in vivo assay for the measurement of intracellular H$_2$O$_2$-associated ROS in C. elegans [36]. The transgenic C. elegans were fed with or without the isoflavones, prior to induction of Aβ-expression, followed by measurement of the levels of H$_2$O$_2$ in the organism. Figure 2A demonstrates that the levels of ROS in the C. elegans CL2006 fed with glycitein for 36 h were reduced (control 100 ± 23%, glycitein 68.9 ± 7 %, n = 3, p = 0.05). Although genistein increased the levels of ROS compared with the untreated controls (Ctrl 100 ± 23 %, genistein 126.1 ± 18 %, n = 3, p = 0.28 total 300 worms in each group), it is not statistically significant. Daidzein did not affect Aβ-induced elevation of ROS (Ctrl 100 ± 23%, daidzein 104.4 ± 6%, n = 6, p = 0.74). These results suggest the decreased Aβ toxicity by glycitein might be, in part, a consequence of its antioxidative action.

To confirm the scavenging effect of glycitein on different species of oxidative free radicals in vitro, we first measured
Scavenging effect of glycitein in the transgenic \textit{C. elegans} and in vitro. \textbf{A}. \( \text{H}_2\text{O}_2 \) level in transgenic \textit{C. elegans} CL4176 fed with different isoflavones. \textit{C. elegans} strain CL4176 was maintained at 16°C for 38 h and then temperature up-shifted to 23°C for 48 h, followed by measurement of \( \text{H}_2\text{O}_2 \) (DCF assay described in methods). CL4176 worms were fed vehicle (Ctrl), 100 \( \mu \)g/ml daidzein, genistein or glycitein from 1 day of age until 3 days of age. At least 60 worms from each group were analyzed for levels of \( \text{H}_2\text{O}_2 \). Results are expressed as a percentage of fluorescence (%DCF) relative to control.

\textbf{B}. Scavenging effect of glycitein on hydroxyl radicals in vitro. The ESR conditions: X-band, 100 kHz modulation with amplitude 1 G, microwave power 10 mW, central magnetic field 3,250 G, sweep width 200 G, temperature 20°C. Inset: ESR spectrum of DMPO-OH generated from Fenton reaction and trapped by DMPO.

\textbf{C}. Scavenging effect of glycitein on superoxide radicals in the system. The ESR conditions are the same as in Fig. 3B. Inset: ESR spectrum of DMPO-OOH generated from Xanthine/xanthine oxidase and trapped by DMPO.

\textbf{D}. Scavenging effect of Soy isoflavone glycitein on \( \cdot\text{CH}_3 \) free radicals in the in vitro system. Inset: ESR spectrum of \( \cdot\text{CH}_3\)-tNB generated from the oxidation of DMSO by ONOO and trapped by tNB.

\textbf{Figure 2}

\textbf{A} Levels of \( \text{H}_2\text{O}_2 \) in \textit{C. elegans} CL2006

\textbf{B} ESR spectrum of hydroxyl radicals

\textbf{C} ESR spectrum of superoxide radicals

\textbf{D} ESR spectrum of peroxynitrite radicals
its effect on hydroxyl radicals. The hydroxyl free radicals were generated from Fenton reaction (H₂O₂ 3%, FeSO₄ 0.1 mM and tapped by DMPO (0.1 mol/l). A spectrum with 4 lines and 1:2:2:1 intensity (g = 2.0045, a₀ = a₁ = 14.9 G) were obtained (Fig. 2B). Figure 2B demonstrates the signal intensity decrease with different concentrations of the soy isoflavone glycitein added into this system. The soy isoflavone glycitein appears to have very strong scavenging effects on hydroxyl radical generated from Fenton reaction (IC₅₀ = 0.035 mg/ml).

We then determined the scavenging effect of glycitein on superoxide free radicals. The superoxide free radicals were generated from xanthine/xanthine oxidase and trapped by DMPO. A signal with 12 lines (a₁₇ = 14.2 G, a₀ = 11.2 G, a₁ = 1.3 G) was obtained (Fig. 2C), and it was decreased with addition of glycitein as shown in Fig. 2C. Apparently, soy isoflavone glycitein has moderate scavenging effect on superoxide free radicals generated from the reaction of xanthine/xanthine oxidase (IC₅₀ = 2 mg/ml).

The reaction of NO with superoxide free radicals is very fast (6.4 × 10⁸mol/L·s⁻¹) and forms peroxynitrite (ONOO⁻). In alkaline solution, it is stable but has a pKa of 6.6 at 0°C and decays rapidly once protonated, to hydroxyl radical-like species and NO₂⁻, which can oxidize sulfhydryls and membrane lipid causing cell toxicity and some diseases. To determine the scavenging effects of the soy isoflavone glycitein on ONOO⁻, the methyl free radical was generated from the oxidation of DMSO by ONOO⁻ and trapped by tNB and a spectrum with 12 lines (a₁₇ = 17.2 G, a₁ = 14.2 G) (Zhao et al. 1996) was obtained (Fig. 2D). A strong scavenging effect of glycitein on ONOO⁻ (IC₅₀ = 0.13 mg/ml) was found as shown in Fig. 2D.

3. \( \beta \) amyloid were significantly reduced in transgenic \( \text{C. elegans} \) fed with glycitein

The modified "amyloid hypothesis" states that \( \beta \)-induced oxidative stress may speed up \( \beta \) amyloid formation and lead to neuronal cell death in AD [37]. To determine whether soy isoflavones affect \( \beta \) amyloid formation in vivo, we measured \( \beta \) amyloid in the transgenic \( \text{C. elegans} \) CL2006 by thioflavin S staining. \( \beta \) amyloid was stained and the fluorescent images were quantified. Quantitatively (Fig. 3), the mean numbers of \( \beta \) amyloid staining per head area of the nematode are significantly reduced only in the transgenic \( \text{C. elegans} \) (CL2006) fed with glycitein (4.1 ± 0.4) compared with unfed controls (6.9 ± 0.5). A moderate reduction, although not significant, was observed in the \( \text{C. elegans} \) fed with genistein (6.1 ± 0.5). No change of \( \beta \) deposits was observed in the worms fed with daidzein (6.9 ± 0.6). None of the three soy isoflavones inhibited \( \beta \) aggregation in vitro (data not shown), suggesting that the decreased \( \beta \) amyloid by glycitein in the transgenic \( \text{C. elegans} \) (Fig. 1) is not due to its direct binding to \( \beta \), but might be a consequence of its antioxidative action (Fig. 2).

### Discussion

In this study, we employed a transgenic \( \text{C. elegans} \) model to evaluate the pharmacological effect of the soy-derived isoflavones genistein, glycitein and daidzein, on \( \beta \)-initiated toxicity and oxidative stress. Results of these assays indicate that among the three isoflavones tested, glycitein delayed \( \beta \) induced paralysis and attenuated the levels of amyloid formation in the transgenic \( \text{C. elegans} \). In
addition, glycitein significantly scavenged hydroxyl free radicals and inhibited the oxidation of peroxynitrite in vitro.

There has been strong evidence for the neuroprotective role of estrogen in aging animal studies and human studies [8,11,26,38-42]. Evidence for estrogen's effect on cognition in women with AD is controversial [10,14]. However, it was reported that ovariectomy in guinea pigs had a pronounced accumulation of β-amyloid plaques compared to intact controls and that estrogen replacement reversed the accumulation [3]. A proposed mechanism for estrogen inhibition of plaque formation is that estrogen induces the cleavage of membranous amyloid precursor protein (APP) generating a soluble proteolytic fragment that precludes the development of β-amyloid plaque formation [5,6]. The possible link between estrogen and Aβ prompted us to determine the effect of phytoestrogens on Aβ-induced toxicity in a model organism. Knowing that apl-1, the member of APP family in C. elegans, lacks a recognizable Aβ sequence [57, 58], the effect of phytoestrogens may have different mechanisms of action. Phytoestrogens have received increasing attention due to their potential protective effects against age-related diseases and hormone-dependent cancers. Phytoestrogens have the ability to selectively activate estrogen receptors, thus affecting many of the biological responses that are caused by endogenous levels of estrogen without concurrent and undesired side effects. Phytoestrogens may act both as an agonist and antagonist in a tissue-specific manner [4]. It was suggested that phytoestrogens can significantly influence sexually dimorphic cognitive behavior by enhancing spatial memory in young adult female animals but inhibit this ability in male [4].

Our observation that glycitein, with weaker estrogenic activities than genistein and daidzein, inhibited Aβ-induced paralysis and deposition, suggests that neuroprotection by phytoestrogens may not be mediated through the estrogenic activity of the compounds. Compared to other soy isoflavones, the estrogenic activity of glycitein is 20 times lower than genistein and daidzein and 200 times lower than 17β estradiol [16]. Soybeans contain large amounts of glycitein and its glycosides, which have been reported to inhibit growth and DNA synthesis of smooth muscle cells [23].

Apparently, it is the antioxidant activity that contributed to the protective effect by glycitein against Aβ-toxicity (Fig. 1) since glycitein is the only soy isoflavone which significantly attenuated the levels of ROS in the C. elegans (Fig. 2). Oxidative free radicals have been postulated as a cause of aging and of some degenerative diseases [45,46]. The formation of free radicals by Aβ in vitro [46] and profound induction of protein carbonyl in the transgenic C. elegans suggests that Aβ-induced oxidative stress triggers Aβ-induced paralysis in the C. elegans [47]. Although Aβ aggregations have been identified as neurotoxic to the brain, oxidative stress is predicted to occur before these aggregations [47] leading to cell apoptosis. Thus, the observed reduction in amyloid formation might also be due to the anti-oxidant activities of glycitein. These observations go along with the free radical hypothesis of aging, which states that there is an imbalance of free radicals and reactive oxygen species (ROS) in the brain causing significant damage to key cellular components [45]. This imbalance may be the causative agent for the pathology of neurodegenerative disorders (such as AD) since most of these disorders are associated with age [48]. The toxicity of free radicals depends on the kinetics of their production, as well as on their stability and transfer efficiency to lipids and proteins. These radicals may interact with other radicals to produce Aβ aggregates [49], and promote the cleavage of the Aβ precursor (APP) supporting the idea that AD can be attributed to continuous oxidative stress, along with a weakened antioxidant status [49].

The causal relationship between ROS and Aβ has been long debated in the field. The transgenic C. elegans would allow us to address the issue. We have conducted a paralysis assay in the C. elegans fed with vitamin C and EGB 761, a Ginkgo biloba leaf extract. Surprisingly, vitamin C alone did not delay Aβ-induced paralysis, but it did when combined with EGB 761, which also inhibits Aβ oligomerization (data not shown), suggesting that it is the combined actions of antioxidants and other protection against Aβ toxicity that is necessary for alleviating Aβ-induced paralysis. Thus, we consider that the antioxidant action is only partially contributing to the protection against Aβ toxicity. Same argument may apply to the discrepancy of the genistein's effect between Fig 2A and Fig 3B; the increased levels of ROS by genistein did not correlate with a decreased Aβ deposition. Defining a functional relationship between Aβ deposition and toxicity, and ROS level is certainly one of our future directions.

The assumption that the protective effect by glycitein against Aβ toxicity might not be mediated by its action on the estrogen receptor is supported by our observation that genistein, with strongest estrogenic activity among soy isoflavones, did not offer protection against Aβ-toxicity. Genistein is a known tyrosine kinase inhibitor. The effective concentration for genistein to activate the estrogen receptor and inhibit tyrosine kinases is much lower (nM-μM) than the concentration we applied to the worms [4] and [5]. These differential concentration effects of genistein might offer protection against Aβ toxicity/paralysis. However, our additional experiments using much lower dosage of genistein did not provide evidence to support this notion. Aβ-induced paralysis was not affected in the
worms fed with either of the two lower concentrations. Since at the given concentration (10 µg/ml, i.e. 37 µM), we observed effects with glycitein but not genistein, we assume that they have differential effects on Aβ-induced paralysis. It has been shown that high dose of genistein (µM) could cause apoptosis in rat primary cortical neurons in vitro via a calcium dependent pathway [43].

We demonstrated a consistent, correlative effect by glycitein against Aβ-induced toxicities using different assays, which suggests that C. elegans is a valid model for mechanistic examination of the transgene products as well as for pharmacological analysis of time course and kinetics of drug effect [50,51]. A relationship between Aβ amino-acid sequence, amyloid formation and oxidative damage was established using this model. Yatin et al. [46] showed both in vitro and in the C. elegans model that methionine (Met[35]) is critical for free radical production by Aβ[1–42], and it is also critical for β-sheet formation in the transgenic C. elegans lines [52]. A correlation between a progressed paralysis phenotype with increased levels of protein carbonyls in CL4176 [47] supports the advanced “amyloid hypothesis” [37]. Mammalian αβ-crystallin (CRYAB) a stress-inducible chaperone protein, which inhibits fibril formation of Aβ-(1–42) [53], has a protein homologue HSP-16 in the C. elegans. This protein has been reported to be colocalized with intracellular Aβ and up regulated in the transgenic Aβ-expression strain of C. elegans [35]. We previously demonstrated that a neuroprotectant, EGb 761, an extract from the ginkgo biloba tree leave, suppressed HSP-16 expression [54]. Although many protein molecules including estrogen receptors are conserved in the nematode [55], the lack of correlation between isoflavone estrogenic activity and suppression of Aβ toxicity in this model system may not exclude the neuroprotection estrogen in AD patients. Nevertheless, it is likely that the temporal sequence of events manifested in the transgenic worms is the same as the one demonstrated in the Drosophila model of AD [56] in that accumulation of Aβ[42] in the brain is sufficient to cause cognitive impairment and neurodegeneration.

Conclusion

We used a transgenic C. elegans model to evaluate the pharmacological effect of the soy-derived isoflavones genistein, glycitein and daidzein, on Aβ-initiated toxicity and oxidative stress. Among the three compounds tested, only glycitein alleviated Aβ expression-induced paralysis in the transgenic C. elegans, which correlated with a reduced level of hydrogen peroxide and β amyloid. These findings suggest that the neuroprotective effect of phytoestrogens is probably due, at least in part, to its antioxidative activities.

Methods

Soy isoflavones were obtained from the National Natural Products Research Center (Oxford, MS). Stock solutions of the soy isoflavones (1 mg/ml, 1000× stock solution) were made in 100% ethanol. The final concentration of ethanol in the food did not exceed 0.01%. DMPO (5,5-dimethyl-1-pyrroline-1-oxide, tNB(3,3,5,5-tetramethyl-pyrroline N-oxide) were purchased from Sigma Chem Co. DMPO was purified by active charcoal.

C. elegans strains

The construction and characterization of the transgenic nematode strains CL2006 and CL4176 have been described previously [34,35]. The CL2006 strain constitutively produces a muscle-specific Aβ[1–42], whereas the expression of Aβ[1–42] in CL4176 depends on a temperature up-shift from 16 to 23°C. Age-synchronized wild type (N2) and the transgenic CL2006 were propagated at 20°C in a temperature-controlled incubator (Sheldon Manufacturing, Model 2005, Cornelius, OR), CL4176 at 16°C, on solid nematode growth medium (NGM) seeded with E. coli (OP50) for food. All chemicals for treatment of experimental animals were added directly to the OP50 food source and began when larvae were 2 days old (for CL2006). In most cases, the nematodes were treated for 4 days (after hatching) with their respective drug. In the life span assay, the C. elegans were treated with the drug for the duration of their lifetime.

Paralysis assays

C. elegans strain CL4176 [35,47] was maintained at 16°C and egg-synchronized onto 35 × 10 mm culture plates containing vehicle or drug. The worms (100 worms on each plate) were allowed to grow for 38 h at 16°C. After 38 hours the temperature was up shifted to 23°C to induce Aβ expression. Paralysis was scored at 1 h intervals until all worms were paralyzed.

H2O2 assay in C. elegans

Intracellular levels of H2O2-related reactive oxidative species (ROS) were measured in C. elegans using 2,7-dichlorofluorescein diacetate (DCF-DA; Molecular Probes). At the end of the specified treatment times, the C. elegans were collected into 100 µl phosphate buffered saline (PBS) (molarity) with 1% Tween-20 (PBST) in eppendorf tubes. The worms were then sonicated (Branson Sonifier 250, VWR Scientific, Suwanee, GA) and pipetted into wells of 96-well plates containing DCF-DA (final concentration 50 µM in PBS). Samples were read every 10 min for 2.5 h. in an FLx800 Microplate Fluorescent Reader (BioTek Instruments, Winooski, VT) at 37°C at excitation 485 nm and emission 530 nm.
ESR assay of free radicals

In order to measure the effect of glycitin on free radicals, the spin trap and the system-generated free radicals were mixed and measured with ESR spectrometer and the signal intensity was taken as H0. Then the system with addition of glycitin was measured again. Hydroxyl radicals (\(H_2O_2 3\%\), \(FeSO_4\) 0.1 mM) and DMPO (0.1 mol/l) were mixed and sucked into a quartz capillary for ESR measurement, and the signal intensity was taken as Hx. The scavenging effect was calculated by \([\frac{\text{Ho-Hx}}{\text{Ho}}] \times 100\%\). The ESR spectra were recorded with Brucker ER200 D-SRC ESR spectrometer. Parameters were employed as follows: X-band, 100 kHz modulation with amplitude 1 G, microwave power 10 mW, central magnetic field 3,250 G, sweep width 200 G, temperature 20°C.

Fluorescent staining and quantitation of \(\beta\) amyloid

Individual CL2006 transgenic nematodes were fixed in 4% paraformaldehyde/PBS, pH 7.4, for 24 h at 4°C, and then permeabilized in 5% fresh \(\beta\)-mercaptoethanol, 1% Triton X-100, 125 mM Tris pH 7.4, in a 37°C incubator for 24 h. The nematodes were transferred, stained with 0.125% thioflavin S (Sigma) in 50% ethanol for 2 min, destained for 2 min in 50% ethanol, washed with PBS and mounted on slides for microscopy. Fluorescence images were acquired using a 40× objective of a fluorescence microscope (BX 60, Olympus, Tokyo, Japan) equipped with a digital camera (Micropublisher 5.0, QIMAGING, Burnaby BC, Canada). The Thioflavin S-reactive deposits anterior of the pharyngeal bulb in individual animals were scored.

Statistical analyses

All statistical tests were performed using a PC-based version of the statistical program Origin 6.0 software (Microcal Software, Inc., Northampton, MA). Statistical comparisons between treatments were done with unpaired student t-test. All figures indicate means and standard error of the mean. Differences with a p value less than 0.05 were defined as statistically significant.

List of abbreviations used

AD, Alzheimer's disease
ROS, reactive oxygen species
\(H_2O_2\), hydrogen peroxide
\(\beta\)B, beta amyloid peptide
ERT, Estrogen Replacement Therapy
SERMs, Selective Estrogen Receptor Modulators

Authors’ contributions

AGZ carried out the paralysis assay, the oxidative stress assay and measurement of levels of ROS. MB conducted some of the oxidative stress assays. ZW performed the fluorescence staining for A\(B\) deposits and the quantitation; JW performed additional experiments for the revision; IK provided the soy isoflavones; CL generated the transgenic C. elegans; BZ participated in the design and analysis of the experiments regarding in vitro assay of ROS; YL participated in the general design of the study, organized collaboration as well as finalizing the manuscript. All authors have read and approved the final manuscript.

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