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Paper

PAPER

Protective effects of baicalin in a *Caenorhabditis elegans* model of Parkinson's disease

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Abstract

Parkinson's disease (PD) is a common neurodegenerative disorder of the central nervous system. However, the pathogenetic mechanisms of PD are far from understood. The aim of this study was to determine the protective effect of baicalin in a *Caenorhabditis elegans* model of PD. *C. elegans* worms were stimulated for 24 h with 6-hydroxydopamine (6-OHDA, 50 mM) and treated with or without baicalin (1, 10, or 100 μM). At all tested concentrations, baicalin improved the reversal and omega turn behavioral phenotypes, as well as the survival, of 6-OHDA-stimulated worms. It also inhibited 6-OHDA-induced oxidative stress by decreasing malondialdehyde levels, increasing superoxide dismutase, glutathione reductase, catalase, and glutathione levels and up-regulating mRNA expression of the antioxidant-related genes *sod-1*, *sod-2*, *sod-3*, *daf-2*, and *daf-16*. Additionally, it significantly decreased the expression of the apoptosis-related gene *ced-3* and increased that of the anti-apoptosis-related gene *ced-9*. The expression levels of cleaved caspase-3 and B-cell lymphoma 2 in 6-OHDA-treated worms were reversed by baicalin. Apoptosis was suppressed by 6-OHDA in loss-of-function strains via the p38 mitogen-activated protein kinase (MAPK) signaling pathway. Furthermore, the apoptotic effects of 6-OHDA were blocked in *sek-1* and *pmk-1* mutants. Finally, the mRNA expression of *sek-1* and *pmk-1* and the protein expression of p38 MAPK and stress-activated protein kinase/extracellular signal-regulated kinase 1 were up-regulated by 6-OHDA and reversed by baicalin. Baicalin may protect against 6-OHDA injury by inhibiting apoptosis and decreasing oxidative stress through the p38 MAPK signaling pathway.

Key words: baicalin, *Caenorhabditis elegans*, 6-hydroxydopamine, oxidative stress, p38 mitogen-activated protein kinase, Parkinson's disease

Introduction

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder characterized by classical motor features and death of nigrostriatal dopamine neurons. It is the second most common neurodegenerative disease worldwide [1]. Characteristic features of PD include selective death of dopamine neurons in the substantia nigra of the midbrain, dopamine deficiency in the basal ganglia, and impairment of cognitive and motor control [2]; however, the pathogenesis of PD is not well understood. There are ongoing studies on treatments for PD; however, there is currently no cure for PD. Several compounds (levodopa, dopamine agonists, and monoamine oxidase B inhibitors) with different mechanisms of action have shown mild efficacy in patients with PD, as they only relieve and delay symptoms of the disease [3]. The most practical treatment for PD that can prevent PD development and considerably alleviate symptoms is yet to be discovered.

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Over the last decade, invertebrate models have been the most used standard laboratory models for studies on PD because they are inexpensive and uncomplicated compared to mammalian models and ethical approval is not required for their use [4]. *Caenorhabditis elegans* (*C. elegans*) has a very well-defined and genetically controllable nervous system and is a proven model for exploring basic mechanisms in the complex human nervous system [5]. It is inexpensive, requires simple growth conditions, and has a simple lifecycle and short generation time. Additionally, *C. elegans* has several genetically altered mutant strains; this allows for simultaneous exploration of the effects of compounds on dopaminergic neurons in different genetic backgrounds and under various environmental conditions [6]. The 6-hydroxydopamine (6-OHDA)-stimulated *C. elegans* model is reliable and stable and can be used to achieve reproducible features that are consistent with the clinical characteristics and pathogenesis of PD [7, 8]. Thus, *C. elegans* with 6-OHDA-induced injury was used as a model of PD in the present study.

Baicalin is a flavonoid in the roots of *Scutellaria baicalensis* Georgi that has been traditionally used to treat several diseases in East Asia since ancient times [9]. It has excellent biological activity and is widely used in the pharmaceutical industry [10]. Recently, it has been reported that baicalin has significant pharmacological effects, such as antitumor, antibacterial, antioxidant, anti-inflammatory, hepatoprotective, and cardioprotective effects [11, 12]. Additionally, baicalin shows a potent neuroprotective effect in various *in vitro* and *in vivo* models of PD. Baicalin attenuates neuronal apoptosis in the substantia nigra of rats with PD via the mammalian target of rapamycin/AKT/glycogen synthase kinase-3b pathway [13]. Furthermore, in a previous study, baicalin improved behavioral performance and reduced dopaminergic neuron loss in the substantia nigra of a PD model and found to be was associated with inhibition of proinflammatory cytokines and oxidative stress [14]. In another study in a *C. elegans* model, baicalin significantly inhibited iron accumulation and had a protective effect on dopaminergic neurons; however, it did not significantly improve 6-OHDA-induced motor dysfunction [15].

We used a *C. elegans* model to investigate the pathogenetic mechanisms responsible for PD. We also determined whether baicalin effectively prevents 6-OHDA-induced injury in *C. elegans* and evaluated the mechanism by which baicalin suppresses the p38 mitogen-activated protein kinase (MAPK) signaling pathway. Overall, the possible therapeutic effect of baicalin in PD was assessed.

Materials and methods

Worm culture

C. elegans N2 (wild-type), *sek-1(km4)* mutants, and *pmk-1(km25)* mutants were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA). Nematodes were cultured using standard methods with few modifications [16]. To avoid contamination with other microbes, *Escherichia coli* OP50 streptomycin-resistant variant strain OP50-1 was used as the food source for the worm strains. All worms were grown at 20°C in nematode growth media (NGM) plates (2.5 mg/mL peptone, 1.7% agar, 50 mM KH_2PO_4 (pH 6.0), 25 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, and 5 µg/mL cholesterol) with fresh OP50-1 as the food source.

Baicalin concentrations

Survival curves were generated for 6-OHDA (10 mM) after treatment for 24 h. Chemicals were purchased from Sigma (Deisenhofen, Germany). Worms were considered dead when no movement response was observed after a gentle touch and/or when there was no pharyngeal pumping. The experiments were performed with approximately 100 worms per treatment in duplicate. Baicalin (1, 10, or 100 µM; Sigma-Aldrich, St Louis, MO, USA) was dissolved in M9 buffer (6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 5 g/L NaCl, and 0.25 g/L $MgSO_4{\cdot}7H_2O$) and administered 30 min before the treatment with 6-OHDA.

Experimental design

L4 *C. elegans* larvae were maintained in NGM agar plates containing OP50-1 at 20 °C. The worms were washed with M9 buffer for three times, transferred into plates containing liquid NGM without OP50, and treated with baicalin or M9 buffer (vehicle). Individual worms (N2, *km4*, and *km25*) were exposed to M9 buffer or baicalin (1, 10, or 100 µM) 30 min before they were treated with 6-OHDA. The worms were exposed to M9 buffer or 6-OHDA (10 mM) for 24 h. The worms were subjected to constant shaking during all phases of treatment for oxygenation in liquid NGM. After 24 h of treatment, the worms were washed with M9 buffer for three times and transferred into NGM agar plates. They were left in the plates for 30 min to acclimate to the environment, after which survival and behavioral assays were performed. Data were obtained from five independent experiments.

Behavioral assays

Omega turn and reversal tests were used to evaluate the behavior of the worms. As previously described, the assays were performed in foodless NGM assay plates (60 mm diameter) [17]. Observations began 1 min after transfer into the plates [18]. Each plate was carefully moved during the assay so that the worms could be observed as they moved across the plate. Omega turns were visually identified as the head nearly touching the tail or a reorientation of 135° within a single head swing. Any backward movement of the entire body was scored as a reversal. A stereo microscope (SZX16; Olympus, Tokyo, Japan) was used to directly observe behavior. Animals were scored by an investigator blinded to the treatments.

RNA extraction and reverse transcription polymerase chain reaction

Reverse transcription polymerase chain reaction (RT-PCR) was performed to verify the differential expression of *sod-1*, *sod-2*, *sod-3*, *daf-2*, *daf-16*, *ced-3*, *ced-9*, *sek-1*, and *pmk-1*. Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions. RNA (5 mg) was used to synthesize the first strand of complementary DNA (cDNA) using SuperScript II RNase H Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and used as a template in subsequent PCR reactions. Thermal cycling was performed using an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany). A NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the concentration of total RNA. Additionally, RNA purity was assessed using the

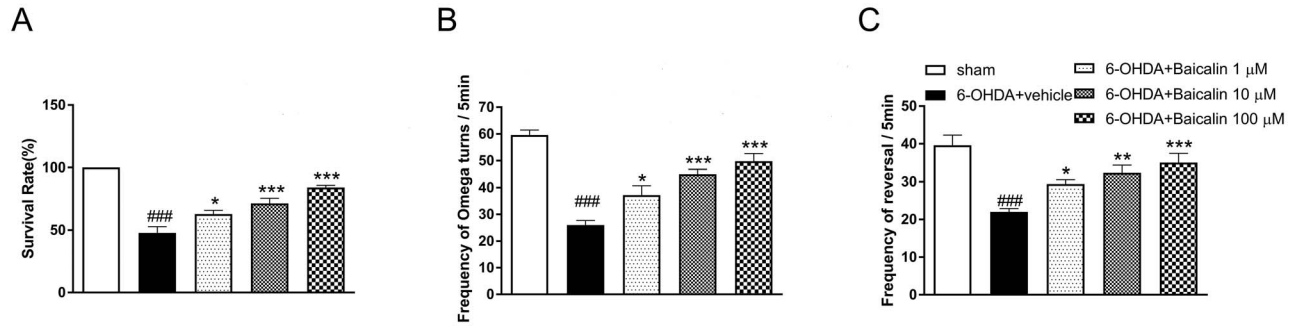

Figure 1: Effects of baicalin on the (a) survival, (b) omega turns, and (c) reversals of *C. elegans*. The worms were used as a model of PD by treating them with 10 mM 6-OHDA. (a) Survival rate and the frequencies of (b) omega turns and (c) reversals were significantly decreased by 6-OHDA but significantly increased by baicalin (1, 10, or 100 μM). Data are presented as mean ± SEM. ### indicates $P < 0.001$ when data are compared to those for the vehicle-treated group. ***, **, and * indicate $P < 0.001$, 0.01, and 0.05, respectively, when data are compared to those for the worms treated with both 6-OHDA and the vehicle.

ratio of optical density (OD) at 260 nm to that at 280 nm. The cDNAs were amplified by RT-PCR using the following primer pairs: *sod-1*: (F) 5′-TGTCGAACCGTGCTGTCGCT-3′, (R) 5′-TGGACCGGCAGAAATGCATCCG-3′; *sod-2*: (F) 5′-ACCATCGGCGGAGTTGCTCA-3′, (R) 5′-AGCGTGCTCCCAGACGTCAA-3′; *sod-3*: (F) 5′-GTGGTGGACACATCAATC-3′, (R) 5′-AAGTGGGACCATTCCTTC-3′; *daf-2*: (F) 5′-GTTGATAATGCTGCCGAG-3′, (R) 5′-ATCCCGGTCCGATTTCTT-3′; *daf-16*: (F) 5′-GGAAGAACTCGATCCGTCACA-3′, (R) 5′-GATTCCTTCCTGGCTTTGCA-3′; *sek-1*: (F) 5′-TGCTCAACGAGCTAGACG-3′, (R) 5′-ATGTTCGACGGTTTCACG-3′; *pmk-1*: (F) 5′-CGACTCCACGAGAAGGAT-3′, (R) 5′-ATATGTACGACGGGCATG-3′; *ced-3*: (F) 5′-ACGGGAGATCGTGAAAGC-3′, (R) 5′-AGAGTTGGCGGATGAAGG-3′; *ced-9*: (F) 5′-AAAGGCACAGAGCCCACC-3′, (R) 5′-CGTTCCCATAACTCGCATC-3′; and β-actin: (F) 5′-CCAGGAATTGCTGATCGTATGCAGAA-3′, (R) 5′-TGGAGAGGGAAGCGAGGATAGA-3′. Cycle number was determined from a linear amplification curve to ensure that amplification was within the linear range. Gene expression was normalized to β-actin expression level. All sample reactions were performed for three times. The relative changes in gene expression were determined using the 2-ΔΔCt method, where $\Delta\Delta Ct = [(Ct_{target} - Ct_{actin})\ \text{sample}] - [(Ct_{target} - Ct_{actin})\ \text{control}]$, using β-actin as the reference gene.

Western blot analysis

C. elegans worms were homogenized in ice-cold lysis buffer containing 50 mM Tris-HCl, 1% NP-40, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid, and 1 mM Na_3VO_4 (pH 7.4) using a Precellys 24 homogenizer (Bertin Technologies, Aix-en-Provence, France). Protein samples were separated on 12% sodium dodecyl sulfate-polyacrylamide gels and electro-transferred onto nitrocellulose membranes. The membranes were blocked with 5% fat-free milk. After that the membranes were incubated with primary antibodies against stress-activated protein kinase/extracellular signal-regulated kinase 1 (Sek-1) (1:1000; Cell Signaling Technology, Danvers, MA, USA), p-p38 (1:1000; Abcam, Cambridge, UK), B-cell lymphoma 2 (Bcl-2, 1:1000, Cell Signaling Technology), c-caspase-3 (1:750, Cell Signaling Technology), and glyceraldehyde 3-phosphate dehydrogenase (1:5000; Kangchen Bio-tech, Shanghai, China) for 2 h at room temperature. Membranes were washed thrice with Tris buffered saline containing Tween 20 and incubated with IRDye700 anti-mouse Molecular Probe (1:3000; LI-COR Biosciences, Lincoln, NE, USA) or IRDye 800 anti-rabbit Molecular Probe (1:8000, LI-COR Biosciences) for 2 h at room temperature. Images were obtained using an Odyssey Infrared Imaging System and analyzed using Odyssey software [19].

Evaluation of antioxidant indices

Malondialdehyde (MDA) levels and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione (GSH) were used as indices of membrane lipid peroxidation and reactive oxygen species (ROS) generation. GSH/MDA content and SOD/GR/CAT activities were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and analyzed using a spectrophotometer. The detailed manipulation processes were performed according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using SPSS 19.0 for Windows (IBM Corp., Armonk, NY, USA). The data were analyzed using one-way analysis of variance followed by the least significant difference or Dunnett's T3 *post-hoc* test for multiple comparisons when equal variances were not assumed. Data have been presented as mean ± standard error of the mean (SEM).

Results

Effects of baicalin on the survival and behavior of 6-OHDA-stimulated *C. elegans*

Treatment with 6-OHDA resulted in a significant decrease in worm survival (47.79%). However, baicalin significantly increased survival (1 μM, 62.92%, $P < 0.05$; 10 μM, 71.40%, $P < 0.001$; and 100 μM, 84.21%, $P < 0.001$) as shown in Figure 1A. The results also showed that 6-OHDA (10 mM) significantly reduced the number of reversals and omega turns in the worms (Fig. 1B), whereas baicalin (1, 10, and 100 μM) significantly improved behavior (Fig. 1C).

Baicalin inhibits apoptosis induced by 6-OHDA in *C. elegans*

The western blotting results showed that the level of cleaved caspase-3 protein was significantly increased in the 6-OHDA-treated group ($P < 0.01$); however, this increased expression was down-regulated by baicalin (1, 10, and 100 μM; Fig. 2A).

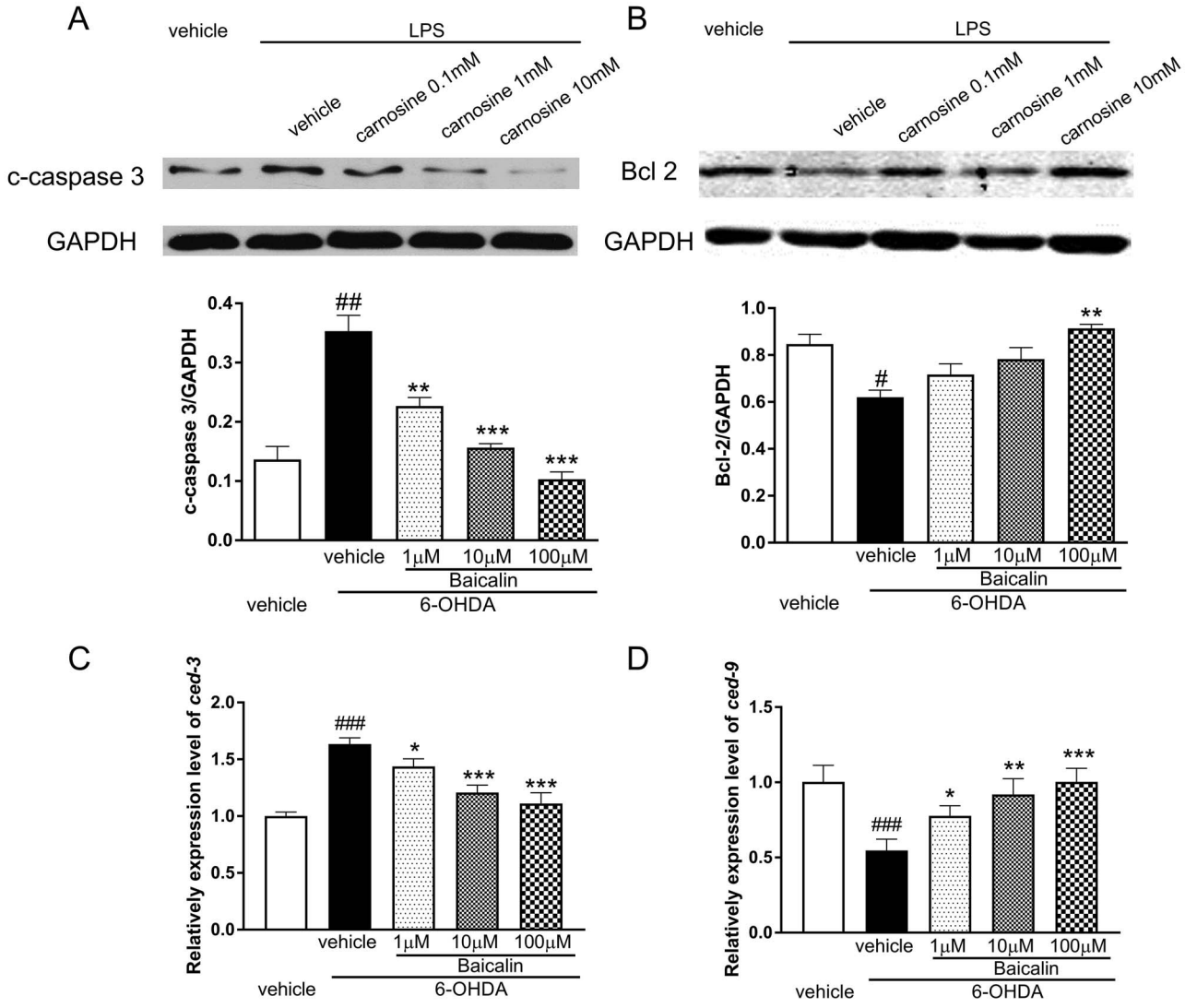

Figure 2: Baicalin ameliorates apoptosis in 6-OHDA-treated *C. elegans*. The levels of (a) c-caspase-3 and (b) Bcl-2 proteins in *C. elegans* were evaluated by western blotting after treating the worms with 6-OHDA. Baicalin significantly reversed 6-OHDA-induced increase in c-caspase-3 and decrease in Bcl-2 levels. Results of the RT-PCR analysis of (c) *ced-3* and (d) *ced-9* gene expression in *C. elegans* after treatment with 6-OHDA. Baicalin effectively reversed 6-OHDA-induced changes in the gene expression of *ced-3* and *ced-9*. Data are presented as mean ± SEM. ###, ##, and # indicate $P < 0.001$, 0.01, and 0.05, respectively, when data are compared to those for the vehicle-treated group. ***, **, and * indicate $P < 0.001$, 0.01, and 0.05, respectively, when data are compared to those for the worms treated with both 6-OHDA and the vehicle.

In contrast, Bcl-2 expression was significantly decreased by 6-OHDA ($P < 0.05$) but increased after the treatment with baicalin in the same group of worms (Fig. 2B). The effects of baicalin on the gene expression of the apoptosis inhibitor *ced-9* (Bcl-2 homolog) and *ced-3* (initiator procaspase) were evaluated via RT-PCR. As shown in Figure 2C and D, the expression of *ced-3* was significantly higher, whereas that of *ced-9* was significantly lower in the 6-OHDA group than in the vehicle group. However, baicalin effectively reversed 6-OHDA-induced changes in the gene expression of *ced-3* and *ced-9*.

Baicalin ameliorates oxidative stress induced by 6-OHDA in *C. elegans*

The levels of MDA, SOD, CAT, GSH, and GR were measured to investigate oxidative stress induced by 6-OHDA and the effects of baicalin in 6-OHDA-treated *C. elegans*. SOD is an enzyme that regulates oxidative stress. The results showed that SOD levels were significantly decreased after the treatment with 6-OHDA ($P < 0.001$). However, baicalin effectively increased SOD levels in the 6-OHDA-treated worms. MDA is a biomarker of oxidative stress. Our findings show that MDA levels were increased by 6-OHDA in the worms ($P < 0.001$); however, this increase was significantly reversed by baicalin. Furthermore, the levels of CAT, GSH, and GR were significantly lower in the 6-OHDA group than in the vehicle group. However, baicalin effectively increased the levels of these enzymes in the worms treated with 6-OHDA (Fig. 3).

Effects of baicalin on up-regulated mRNA expression of stress- and longevity-related genes

The mRNA expression of *sod-1* in *C. elegans* decreased in response to 6-OHDA ($P < 0.001$, Fig. 4A); however, this was significantly reversed by baicalin. The mRNA levels of *sod-2* and *sod-3* were

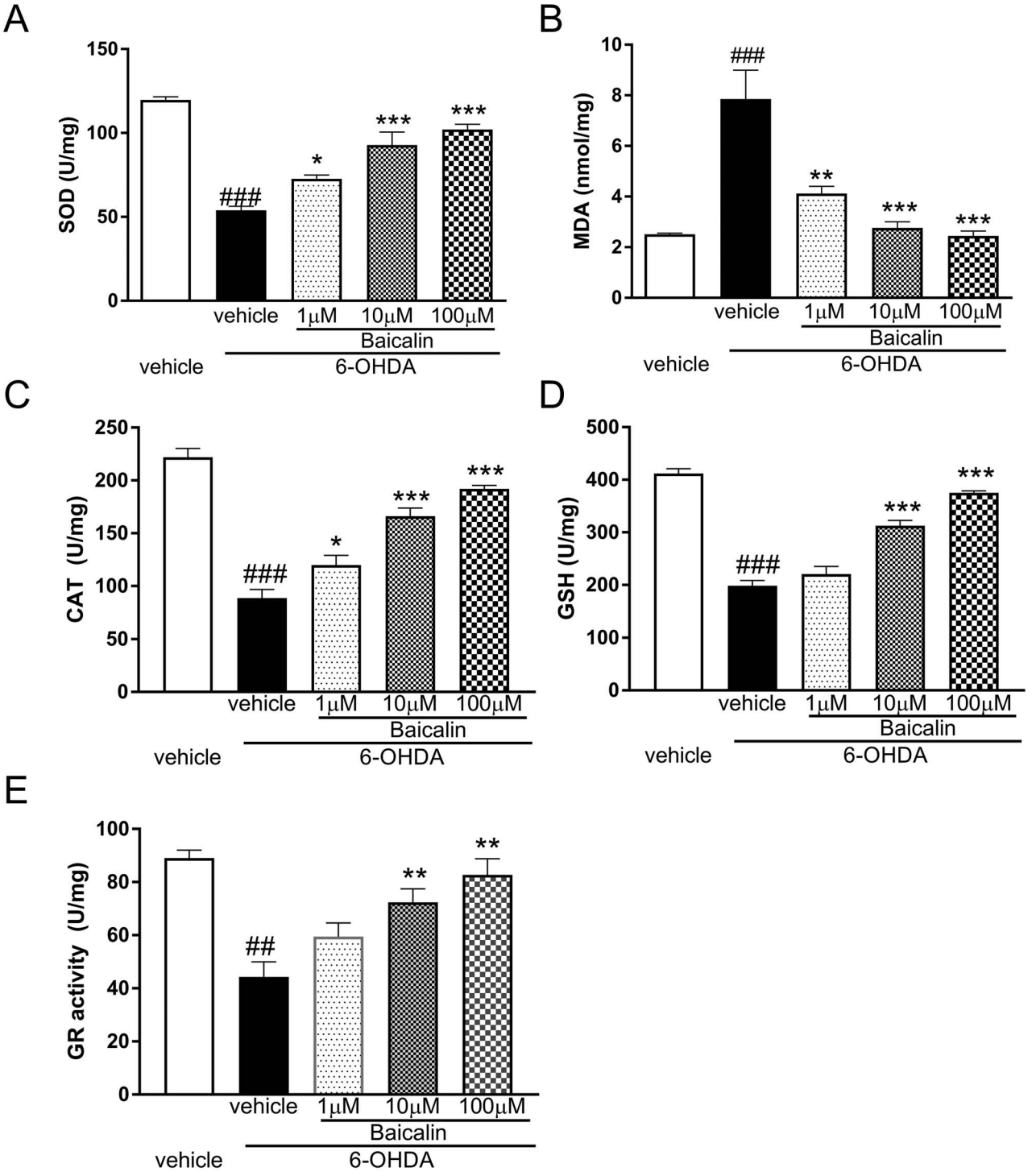

Figure 3: Baicalin ameliorates oxidative stress in 6-OHDA-treated *C. elegans*. The levels of (a) SOD, (b) MDA, (c) CAT, (d) GSH, and (e) GR were measured after the worms were treated with 6-OHDA. Baicalin significantly reversed 6-OHDA-induced changes in the levels of the enzymes. Data are presented as mean ± SEM. ### and ## indicate $P < 0.001$ and 0.01, respectively, when data are compared to those for the vehicle-treated group. ***, **, and * indicate $P < 0.001$, 0.01, and 0.05, respectively, when data are compared to those for the worms treated with both 6-OHDA and the vehicle.

also significantly decreased in response to 6-OHDA ($P < 0.001$); however, pretreatment with baicalin resulted in up-regulation of the expression of these genes (Fig. 4B and C). PCR was performed to investigate the effects of baicalin on *daf-2* and *daf-16* gene expression. The results revealed that *daf-2* expression in *C. elegans* increased following treatment with 6-OHDA ($P < 0.01$). However, this increase was significantly reversed by baicalin at concentrations of 1, 10, and 100 μM (Fig. 4D). In contrast, the expression level of *daf-16* significantly decreased in response to 6-OHDA ($P < 0.01$), although pretreatment with 10 or 100 μM baicalin resulted in increased *daf-16* expression in the 6-OHDA-treated worms (Fig. 4E).

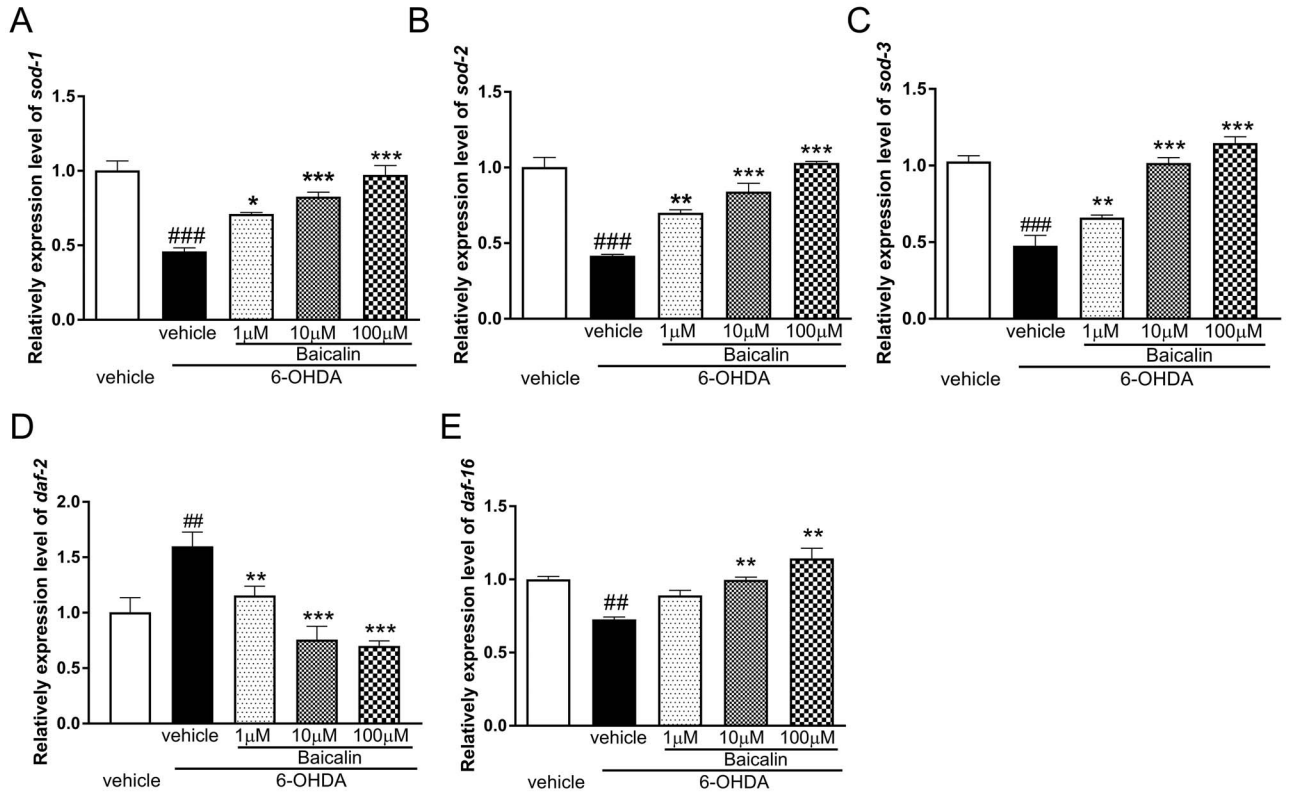

Figure 4: Effects of baicalin on the mRNA expression levels of (a) *sod-1*, (b) *sod-2*, (c) *sod-3*, and (d) *daf-16* in 6-OHDA-treated *C. elegans*. The mRNA levels of these genes were decreased by 6-OHDA; however, the decreases were significantly reversed by baicalin. Data are presented as mean ± SEM. ## indicates $P < 0.01$ when data are compared to those for the vehicle-treated group. ** and * indicate $P < 0.01$ and 0.05, respectively, when data are compared to those for the worms treated with both 6-OHDA and the vehicle.

Effects of baicalin on the p38 MAPK signaling pathway in *C. elegans* treated with 6-OHDA

As shown in Figure 5A, survival of the *pmk-1(km25)* and *sek-1(km4)* mutants was not affected by 6-OHDA, suggesting that *pmk-1* and *sek-1* may play pivotal roles in mediating 6-OHDA-induced death of *C. elegans*. The transcriptional expression of *pmk-1* and *sek-1* was significantly higher in the worms treated with 6-OHDA for 24 h than in the vehicle-treated worms (Fig. 5B and C). However, baicalin effectively reduced *pmk-1* and *sek-1* gene expression in 6-OHDA-treated worms. The levels of phosphorylated Sek-1 and p-p38 were measured to determine the effects of baicalin on the p38 MAPK signaling pathway. The expression levels of both Sek-1 and p-p38 were greatly increased in the 6-OHDA-treated worms but reduced following treatment with baicalin (Fig. 5D and E).

Discussion

In this study, we used an invertebrate model of 6-OHDA-induced PD to evaluate the effects of baicalin in PD for the first time. We found that 6-OHDA caused significant mortality of the nematodes and reduced the frequencies of reversals and omega turns. However, baicalin increased the frequencies of reversals and omega turns and survival rate. Our present findings also show that baicalin significantly attenuated oxidative injury and prevented apoptosis. It is likely that the p38 MAPK signaling pathway is associated with the protective effect of baicalin.

Baicalin has been reported to show significant pharmacological effects [11]. However, reports on its effects against 6-OHDA-induced injury in *C. elegans* are rare. In the present study, we observed that baicalin significantly improved the survival and behavioral phenotype of 6-OHDA-treated *C. elegans*, suggesting that baicalin may have clinical value in the treatment of 6-OHDA-induced injury. Baicalin ameliorated 6-OHDA-induced oxidative stress, which was evidenced by its significant inhibition of 6-OHDA-induced increases in MDA level and decreases in SOD, GR, CAT, and GSH levels. The antioxidative effect of baicalin was confirmed by its reversal of the up-regulated mRNA expression of *sod-1*, *sod-2*, *sod-3*. Oxidative stress plays an important role in the complex cascade underlying dopaminergic neurodegeneration in PD [20, 21]. Research has shown that oxidative stress is a significant feature of the initial stage of PD [22]. Our results further confirm the potential protective effect of baicalin on PD. For the first time, we found that baicalin significantly reversed the decrease in in *daf-16* expression and increase in *daf-2* expression that were induced by 6-OHDA. The insulin/insulin-like growth factor-1 transmembrane receptor ortholog *daf-2* is associated with oxidative stress response and lifespan [23]. Furthermore, the transcription factor *daf-16* is a homologous gene of mammalian forkhead box O proteins in *C. elegans* that plays a critical role in stress response [24]. *Daf-16*-knockout worms are highly sensitive to oxidative stress and have short lifespans [25]. These results indicate that baicalin may prolong the lifespan in PD.

We also found baicalin effectively increased Bcl-2 expression and reduced caspase-3 levels in the worms treated with 6-OHDA. In addition, baicalin effectively reversed the decrease in *ced-9* (a homolog of mammalian Bcl-2) expression and increase in

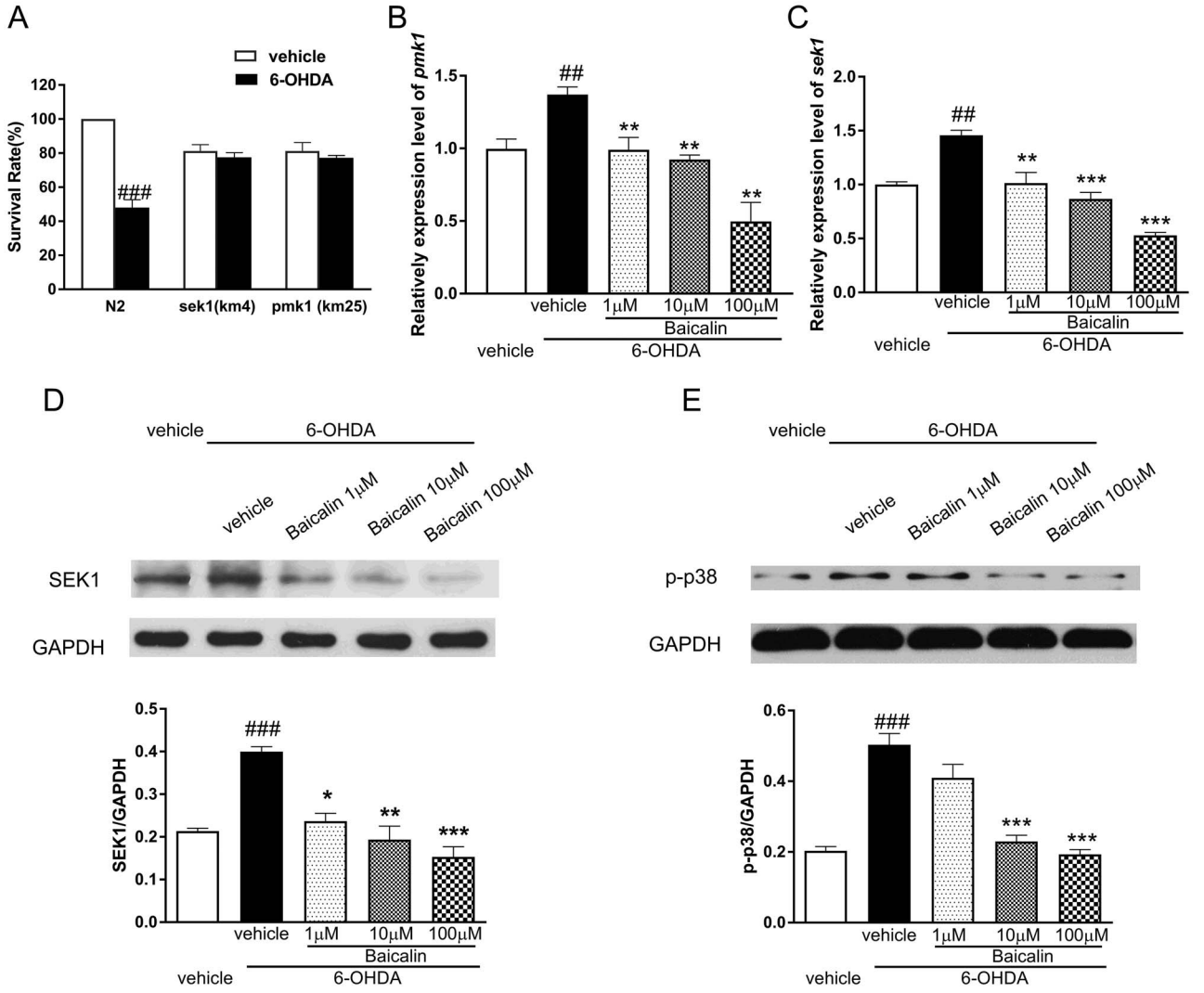

Figure 5: Effects of baicalin on the expression of proteins and genes involved in the p38 MAPK signaling pathway in *C. elegans*. (a) The survival rates of *pmk-1(km25)* and *sek-1(km4)* mutants were not changed after treatment with 6-OHDA. The mRNA levels of (b) *pmk-1* and (c) *sek-1* were determined by RT-PCR. The protein levels of (d) Sek-1 and (e) p-p38 were determined by western blotting after treating the worms with 6-OHDA. Data are presented as mean ± SEM. ### and ## indicate $P < 0.001$ and 0.01, respectively, when data are compared to those for the vehicle-treated group. ***, **, and * indicate $P < 0.001$, 0.01, and 0.05, respectively, when data are compared to those for the worms treated with both 6-OHDA and the vehicle.

ced-3 (a homolog of mammalian caspase-3) expression that were induced by 6-OHDA. Apoptosis plays a key role in the progression of PD [26]. Cell death and neurodegenerative conditions have been linked to imbalance between generation of free radicals and antioxidant defenses and oxidative stress [27, 28]. Some antioxidants have been reported to protect dopaminergic neurons from apoptosis by antioxidation in PD [29, 30]. Human umbilical cord mesenchymal stromal cell also has been reported to protect the apoptosis by reducing oxidative stress in PD [31]. Lactoferrin (a non-heme iron-binding glycoprotein) protects against oxidative stress and apoptosis in PD mice [32]. Our results show that 6-OHDA-induced apoptosis in *C. elegans* could be attenuated by anti-oxidation of baicalin, which further demonstrated the potential protective effect of baicalin on PD.

It has been reported that p38 MAPK plays a key role in PD [33]. Additionally, p38, as a member of the MAPK superfamily, is activated by various cellular stresses and ligands. Therefore, we investigated the function of the p38 MAPK signaling pathway in the regulation of 6-OHDA toxicity in *C. elegans* with mutations in *pmk-1(km25)* and *sek-1(km4)*, which are genes associated with the p38 MAPK signaling pathway in nematodes. This is because *pmk-1* is a p38 MAPK homolog associated with apoptotic regulation of germ cells, innate immune response, and oxidative reactions in *C. elegans* [34, 35]. Furthermore, *sek-1* is an MAPK and the *C. elegans* ortholog of mammalian MKK3/MKK6 in the p38 MAPK pathway that is required for innate immunity in *C. elegans* [36, 37]. Apoptosis was suppressed in the loss-of-function strains via the p38 MAPK signaling pathway after treatment with 6-OHDA. However, the apoptotic effects of 6-OHDA were blocked in the *sek-1* and *pmk-1* mutants. Our results from both the RT-PCR and western blot analyses demonstrated that 6-OHDA significantly increased the transcriptional expression of *pmk-1* and *sek-1* and the protein expression of p-p38 and Sek-1. However, the p38 MAPK signaling pathway was inhibited after treatment with baicalin, indicating that baicalin might act as a suppressor of the p38 MAPK signaling pathway.

In conclusion, the results of our investigations on the effects of baicalin in 6-OHDA-treated *C. elegans* show that baicalin may

protect against PD. Our findings show that baicalin protected the worms against 6-OHDA-induced injury by inhibiting apoptosis and decreasing oxidative stress via the p38 MAPK signaling pathway. Therefore, baicalin may be a useful adjunctive treatment in the management of PD; however, further studies on the detailed mechanisms underlying the effects of baicalin are needed.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

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