Beneficial effects of *Glycyrrhiza radix* extract in preventing oxidative damage and extending the lifespan of *Caenorhabditis elegans*

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**Abstract**

**Ethnopharmacological relevance:** *Glycyrrhiza radix* (GR) is a medicinal herb extensively used in traditional Chinese medicine. This study aimed to evaluate the pharmacological effect of GR and the possible mechanisms of GR, to provide a pharmacological basis in traditional medicine.

**Materials and methods:** In the present study, *C. elegans* (L1-larvae to young adults) was exposed to 0.12–0.24 g/mL of GR in 12-well sterile tissue culture plates at 20 °C in the presence of food. Lethality, growth, lifespan, reproduction, locomotion, metabolism, intestinal autofluorescence, and reactive oxygen species (ROS) production assays were performed to investigate the possible safety profile and beneficial effects of GR in these nematodes. We found that the lifespan of nematodes exposed to 0.18–0.24 g/mL of GR was extended. We then determined the mechanism of the longevity effect of GR using quantitative reverse transcription PCR and oxidative stress resistance assays induced by heat and paraquat.

**Results:** Prolonged exposure to 0.12–0.24 g/mL of GR did not induce lethality, alter body length, morphology or metabolism, affect brood size, locomotion, the development of D-type GABAergic motor neurons, or induce significant induction of intestinal autofluorescence and intestinal ROS production. In *C. elegans*, pretreatment with GR suppressed the damage due to heat-stress or oxidative stress induced by paraquat, a ROS generator, on lifespan, and inhibited the induction of intestinal ROS production induced by paraquat. Moreover, prolonged exposure to GR extended lifespan, increased locomotion and decreased intestinal ROS production in adult day-12 nematodes. Furthermore, prolonged exposure to GR significantly altered the expression patterns of genes encoding the insulin-like signaling pathway which had a key role in longevity control. Mutation of *daf-16* gene encoding the FOXO transcription factor significantly decreased lifespan, suppressed locomotion, and increased intestinal ROS production in GR exposed adult nematodes.

**Conclusions:** GR is relatively safe and has protective effects against the damage caused by both heat-stress and oxidative stress at the examined concentrations. Furthermore, GR is capable of extending the lifespan of nematodes, and the insulin-like signaling pathway may play a crucial role in regulating the lifespan-extending effects of GR.

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1. Introduction

*Glycyrrhiza radix* (GR) is a medicinal herb extensively used in traditional Chinese medicine formulae for coordinating the actions of various components in the recipes and strengthening body functions (Chan et al., 2006). This herb is usually used with other traditional Chinese medicine in clinical practice, mainly for the treatment of peptic ulcer, hepatitis C, and pulmonary and skin diseases (Asl and Hosseinzadeh, 2008; Liu, 2009; Jin et al., 2010; Chang and Li, 2014; Mao et al., 2014). Pharmacology study showed that GR has various pharmacological properties. GR extract has significant inhibitory effects on LPS-induced nitric oxide production in mouse macrophage RAW264.7 cells, suggesting that it has anti-inflammatory activity (Yue et al., 2012). GR extract may...
contain active components which modulate the immune response by influencing intracellular signaling pathways (Li et al., 2012; Chan et al., 2006). GR extract showed potent attenuation of acetylcholine- and carbachol-induced contractions in rat trachea, indicating its relaxant activity (Yue et al., 2012). The flavonoid extracts from GR can inhibit hepatitis C virus replication (Sekine-Osajima et al., 2009).

Previous in vitro and in vivo studies have demonstrated the protective effects of GR extract and its active components on organisms (Yokozawa et al., 2000; Yokozawa et al., 2005; Lee et al., 2012). GR extract may ameliorate hypoxia (ischemia)-reoxygenation (reperfusion) injury and improved renal function (Yokozawa et al., 2000). GR extract has cytoprotective effects against cadmium-induced toxicity (Kim et al., 2006), and can protect the kidneys against ONOO- by scavenging ONOO- and/or its precursor NO (Yokozawa et al., 2005). GR extract and its active component, isoliquiritigenin, inhibited Aβ-induced neuronal apoptotic death and ROS production in cultured cortical neurons (Lee et al., 2012). However, GR has many different components, and some of these components may have adverse effects on organisms. For example, liquiritigenin, an active component in GR, induced ROS production and apoptosis in SMMC-7721 cells (Zhang et al., 2009). Therefore, the safety profile of GR extract should be carefully evaluated before use.

The free-living nematode Caenorhabditis elegans is a widely studied model animal (Brenner, 1974; Zhao and Wang, 2012). C. elegans has been studied in many aspects of biology, such as longevity (Shen et al., 2010; Wang et al., 2013). C. elegans is also considered to be an excellent animal for toxicity testing as it offers a system best suited for in vivo study (Leung et al., 2008; Zhao et al., 2013). C. elegans is effectively used in the toxicological study of many toxicants including metals (Wang et al., 2010; Liu et al., 2012; Song et al., 2014), organic pollutants (Ruan et al., 2012; Zhuang et al., 2014), engineered nanomaterials (Li et al., 2013a, 2013b, 2013c; Rui et al., 2013; Nouara et al., 2013; Wu et al., 2013a, 2013b, 2013c; Ruan et al., 2012), and drugs (Ye et al., 2008; Kumar et al., 2010; Li et al., 2013a, 2013b, 2013c). In particular, several studies have shown that C. elegans can be used to investigate the beneficial or adverse effects of the components of specific plants or Chinese medicines (Liu et al., 2013; Rui et al., 2009; Dostal et al., 2010; Fan et al., 2011; Sangha et al., 2012; Yang et al., 2012; Zhang et al., 2013a, 2013b).

In the present study, we used C. elegans in a prolonged exposure assay to assess the in vivo toxicity of GR extract, to evaluate the pharmacological effect of GR and to determine the possible mechanisms involved in the effect of GR.

2. Materials and methods

2.1. Plant material and chemicals

Dried GR was collected from the Meikang licorice planting base in Lingwu city, Ningxia province, China. No specific permissions were required to collect the plants, as the location does not belong to any national park or protected area of land or sea. We also confirmed that the area did not involve endangered and protected species. GR was authenticated and the voucher specimen (No. 110326) has been deposited at the Herbarium in Jiangsu Key Laboratory for High Technology Research of Traditional Chinese Medicine Formulae, Nanjing University of Chinese Medicine. The raw-processed GR (72 g) was decocted twice with 720 mL of boiling distilled water at 80 °C for 2 h. The decocted suspensions were filtered using qualitative filter papers (medium speed), collected, and concentrated by decompressive rotary evaporation. The final concentration of the concentrated solution was 0.72 g/mL GR (equivalent to the dry weight of raw materials). The concentrated solutions were stored at −20 °C until use.

Our group undertakes the research on the incompatibility mechanism for Flos Genkwa and Glycyrrhizae. The previous study by our group found that Flos Genkwa at concentrations of 0.12–0.24 g/mL significantly increased the mean defecation cycle length, Flos Genkwa at concentrations of 0.18–0.24 g/mL significantly decreased the function of reproduction and locomotion, increased the mean defecation cycle length, shortened the body length (Qiao et al., 2014). In addition, the previous study showed that the toxicity of Flos Genkwa co-decoction with Glycyrrhizae by the ration of 1:1 was higher than the toxicity of Flos Genkwa (Wang et al., 2014). Considering the following research mainly on the toxicity of Flos Genkwa co-decoction with Glycyrrhizae by different ration, 0.12, 0.18, 0.24 g/mL were selected as the exposed concentration of Glycyrrhizae finally.

In test, the concentrated solution was heated in water at 37 °C for 30 min, shaken well, and progressively diluted with sterile M9 solution to 0.24, 0.18, 0.12 g/mL. So the test solutions were uniform. The concentrations of the test solutions were 0.12, 0.18, 0.24 g/mL, respectively. All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Strain preparation

The nematodes used in the present study were wild-type N2, daf-16(mu86) mutant, and the transgenic strain of oxis12[Ex(Epc-47:GFP)], originally obtained from the Caenorhabditis Genetics Center (CGC). They were maintained in nematode growth medium (NGM) plates seeded with Escherichia coli OP50 at 20 °C as described previously (Brenner, 1974). Age synchronous populations of L1-larvae nematodes were obtained as described previously (Donkin and Williams, 1995). Exposures were performed from L1-larvae to young adult in 12-well sterile tissue culture plates at 20 °C in the presence of food. Age synchronous populations of L1-larvae nematodes were picked into GR test solution until they grow up to young adult. Young adult were picked out to new fresh NGM and observed in the following assays.

2.3. Lethality and growth assays

For the lethality assay, a 1.0 mL aliquot of the test solution was added to each well of a tissue culture plate, which was subsequently loaded with 50 nematodes for each treatment. Following exposure, the wells were observed under a dissecting microscope, and inactive nematodes were scored. Three replicates were examined per treatment.

After exposure to GR test solution, growth was assessed by body length, which was determined by measuring the flat surface area of adult nematodes using Image-Pro® Express software. Twenty nematodes were examined per treatment.

2.4. Lifespan assay

For the lifespan assay, the assay was performed as described previously (He et al., 2009; Wang et al., 2010). After exposure to GR test solution, the hermaphrodites were transferred daily for the first 4 days of adulthood. Nematodes were observed every day and were scored as dead when they did not move even after repeated taps with a pick. Thirty nematodes were examined per treatment. For lifespan, graphs were representative of three trials.

2.5. Reproduction and locomotion behavior assays

After exposure to GR test solution, reproduction assay was performed as described previously (Wang and Xing, 2010, 2009). A
single control or exposed N2 nematode was placed onto an NGM plate with OP50. Each day, all P0 nematodes were transferred to a new NGM plate. To assay brood size, the numbers of offspring at all stages beyond the egg were counted. Twenty nematodes were examined per treatment.

Locomotion behavior assay was performed as described previously (Wang and Xing, 2010, 2009). Locomotion behavior was evaluated by endpoints of both head thrash and body bend. Head thrash was defined as a change in the direction of bending at the mid body. After exposure to GR test solution, every animal was transferred into a microtiter well containing 60 μL of K medium on the top of agar. After a 1 min recovery period, head thrashes were counted for 1 min. Thirty nematodes were examined per treatment.

Body bend was counted as a change in the direction of the part of nematodes corresponding to the posterior bulb of the pharynx along the y-axis, assuming that nematode was traveling along the x-axis. After exposure to GR test solution, animals were picked onto a new plate without food and scored for the number of body bends in an interval of 20 s. Thirty nematodes were examined per treatment.

2.6. Pumping rate and mean defecation cycle length

These measurements were performed as described previously (Wu et al., 2012a, 2012b; Zhao et al., 2014). To assay the pumping rate, after exposure to GR test solution, nematodes were placed on NGM plates with food, and left undisturbed for 1 h before measuring. Pharyngeal pumping was counted for 1 min under DIC optics using a Zeiss microscope. To assay mean defecation cycle length, images were photographed and examined on the same day to avoid the effects of light source variance on fluorescent intensity. Thirty nematodes were examined per treatment.

2.7. Intestinal autofluorescence and ROS production

Photographs of autofluorescence were obtained as described previously (Wu et al., 2012a, 2012b; Wu et al., 2011a, 2011b). The images were collected for endogenous intestine fluorescence using a 525-nm bandpass filter and without automatic gain control in order to preserve the relative intensity of fluorescence in different animals. Observations of autofluorescence were recorded and color images were obtained for documentation of the results with Magnafire® software (Olympus, Irving, TX, USA). Lipofuscin levels were measured using ImageJ Software (NIH Image) by determining average pixel intensity in the intestine of each animal. Twenty nematodes were included for the statistical analysis.

After exposure to GR test solution, the examined nematodes were transferred to 1 mL of M9 buffer containing 1 μM CM-H2DCFDA in 12-well sterile tissue culture plates and pre-incubated for 3 h at 20 °C, and then mounted on 2% agar pads for examination with a laser scanning confocal microscope (Leica, TCS SP2, Bensheim, Germany) at 488 nm of excitation wavelength and 510 nm of emission filter. The relative fluorescent intensities of the intestines were semi-quantified, and the semi-quantified ROS were expressed as relative fluorescent units (RFU). Twenty nematodes were examined per treatment.

2.8. Quantitative real-time polymerase chain reaction (PCR)

After exposure to GR test solution, the total RNA was extracted using the RNeasy Mini Kit (Qiagen). Total nematode RNA (∼1 μg) was reverse-transcribed using a cDNA Synthesis kit (Bio-Rad Laboratories). Quantitative reverse transcription PCR (RT-PCR) was run at the optimized annealing temperature of 58 °C. The relative quantification of targeted genes in comparison to the reference tba-1 gene encoding the tubulin protein was determined. The final results were expressed as the relative expression ratio (between the targeted gene and reference gene). The designed primers for targeted genes and the reference tba-1 gene are shown in Table S1.

2.9. Analysis of axonal degeneration and neuronal loss of D-type GABAergic motor neurons

This method was performed as described previously (Zhao et al., 2014). D-type GABAergic motor neurons were visualized using a transgenic strain of oxis12. Numbers of ventral and dorsal cord gaps were quantified to reflect axonal degeneration. Neuronal loss was examined by counting the number of cell bodies in the D-type GABAergic nervous system. After exposure to GR test solution, images were photographed and examined on the same day to avoid the effects of light source variance on fluorescent intensity. Thirty nematodes were examined per treatment.

2.10. Heat stress and oxidative stress resistance assays

These assays were performed as described previously (Zhang et al., 2013a, 2013b). Nematodes pre-treated with GR for 48 h were transferred to the 35 °C condition for 16 h or 2 mM of paraquat, a ROS generator (Liu et al., 2012), for 6 h, and then the lifespan was analyzed as described above.

2.11. Analysis of the main chemical components in raw-processed GR

To analyze the chemical components of GR, reverse phase high-performance liquid chromatography (RP-HPLC) was performed to establish the chromatographic fingerprint. The separation was performed on an Elite C18 column (4.6 mm × 250 mm, 5 μm) with a mobile phase composed of methanol (A) and 0.25% acetic acid (B). The gradient elution condition was 0 min, 20% (A); 30 min, 60% (A); 45 min, 100% (A); 50 min, 20% (A). The column temperature was set at 30 °C and the flow rate was 1.0 mL/min. The detection wavelength was set at 260 nm. The injection volume was 10 μL. Comparing the HPLC retention times and AU of target peaks with those of the standards, we determined the main components of the prepared GR.

2.12. Statistical analysis

All data were expressed as means ± standard error of the mean (S.E.M.). Graphs were generated using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Statistical analysis was performed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Differences between groups were determined using analysis of variance (ANOVA). Probability levels of 0.05 and 0.01 were considered statistically significant.

3. Results

3.1. Effects of GR exposure on survival and development of nematodes

In the in vivo assay system, we first performed a safety evaluation in nematodes exposed to the examined concentrations of GR. Considering the long-term application of Chinese medicines; we assessed prolonged exposure to GR in C. elegans L1-larvae to young adults. Following prolonged exposure to GR at concentrations of 0.12–0.24 g/mL, we did not observe the induction of lethality (Fig. 1A). Similarly, prolonged exposure to 0.12–0.24 g/mL...
of GR did not significantly alter body length (Fig. 1B). In addition, prolonged exposure to 0.12–0.24 g/mL of GR did not obviously influence the morphology of nematodes (Fig. 1C). These results suggest that prolonged exposure to GR at the examined concentrations did not affect the survival and development of nematodes.

3.2. Effects of GR exposure on metabolism of nematodes

Pumping rate and mean defecation cycle length can serve as important endpoints in the evaluation of metabolism in nematodes (Wu et al., 2013a, 2013b, 2013c). Interestingly, we found that prolonged exposure to GR at concentrations of 0.12–0.24 g/mL did not significantly alter the pumping rate of nematodes (Fig. 1D). Similarly, prolonged exposure to GR at concentrations of 0.12–0.24 g/mL did not obviously affect the mean defecation cycle length of nematodes (Fig. 1E). These data indicate the relatively normal state of metabolism in nematodes exposed to the examined concentrations of GR.

3.3. Effects of GR exposure on the functions of secondary targeted organs in nematodes

In nematodes, reproductive organs and neurons are possibly important secondary targeted organs for drugs or toxicants (Wu et al., 2013a, 2013b, 2013c). We next investigated the effects of prolonged exposure to GR on reproduction using brood size as the endpoint. Prolonged exposure to 0.12–0.24 g/mL of GR did not noticeably influence the brood size of nematodes (Fig. 2A). In addition, we found that prolonged exposure to 0.12–0.24 g/mL of GR did not significantly decrease either head thrash or body bend in nematodes (Fig. 2B and C). Our data demonstrate the normal physiological state of possible secondary targeted organs in nematodes exposed to the examined concentrations of GR.

3.4. Effects of GR exposure on the development of D-type GABAergic motor neurons in nematodes

In C. elegans, locomotion behavior is under the control of D-type GABAergic motor neurons (McIntire et al., 1993). Prolonged exposure to 0.12–0.24 g/mL of GR did not obviously alter the morphology of D-type GABAergic motor neurons (Fig. 2D). Prolonged exposure to 0.12–0.24 g/mL of GR did not induce obvious neuronal loss (Fig. 2E) and deficits in axonal development (Fig. 2F). Thus, GR exposure at the examined concentrations did not have the potential to cause damage to the development of D-type GABAergic motor neurons in nematodes.

3.5. Effects of GR exposure on functions of primary targeted organs in nematodes

In nematodes, the intestine is the primary targeted organ for drugs or toxicants (Wu et al., 2013a, 2013b, 2013c). We investigated the effects of prolonged exposure to GR on the function of the intestine in nematodes exposed to GR. Intestinal autofluorescence caused by lysosomal deposits of lipofuscin can
accumulate over time in aging nematodes or nematodes exposed to toxicants (Wu et al. 2011a, 2011b; Shen et al., 2010). After prolonged exposure, GR at concentrations of 0.12–0.24 g/mL did not significantly induce intestinal autofluorescence compared with controls (Fig. 3A). Moreover, we found that prolonged exposure to 0.12–0.24 g/mL of GR did not cause significant induction of intestinal ROS production compared with controls (Fig. 3B). Therefore, the normal physiological state for both the primary and the secondary targeted organs can be sustained in nematodes exposed to the examined concentrations of GR.

3.6. Heat-stress resistance property of GR in C. elegans

To investigate whether GR has a stress resistance property, nematodes pretreated with 0.24 g/mL of GR for 48 h were further exposed to heat-stress (35 °C) for 16 h. After 0.24 g/mL of GR pretreatment, nematodes exhibited significant resistance to heat-stress (Fig. 4). Pretreatment with 0.24 g/mL of GR effectively increased the lifespan of nematodes treated with heat-stress at 35 °C for 16 h (Fig. 4A), which demonstrated the heat-stress resistant property of GR treatment in
nematodes.

3.7. Oxidative resistance property of GR in *C. elegans*

To further investigate whether GR has oxidative stress resistance properties, nematodes pretreated with 0.24 g/mL of GR for 48 h were exposed to 2 mM of paraquat for 6 h. Following 0.24 g/mL of GR pretreatment, the nematodes exhibited significant resistance to oxidative stress induced by paraquat (Fig. 4B and C). Pretreatment with 0.24 g/mL of GR significantly increased the lifespan of nematodes treated with paraquat (Fig. 4B). Moreover, pretreatment with 0.24 g/mL of GR significantly inhibited the induction of intestinal ROS production induced by 2 mM of paraquat in nematodes (Fig. 4C). These data suggest that pretreatment with GR induced resistance properties in nematodes against damage from both heat-stress and oxidative stress.

3.8. Beneficial effects of GR exposure on lifespan and age-related properties in nematodes

In addition to protective effects, we also found lifespan-extending effects with GR treatment in nematodes. After prolonged exposure, although 0.12 g/mL of GR did not significantly affect lifespan, 0.18–0.24 g/mL of GR significantly increased the lifespan of nematodes compared with controls (Fig. 5A).

Previous studies showed that some drugs with lifespan-extending properties can also alter age-related properties in *C. elegans* (Zhang et al., 2013a, 2013b). In adult day-12 nematodes, we observed the induction of intestinal ROS production and a decrease in locomotion behavior (Zhang et al., 2013a, 2013b). We observed that prolonged exposure to 0.24 g/mL of GR significantly suppressed the induction of intestinal ROS production in adult day-12 nematodes compared with controls (Fig. 5B). Moreover, prolonged exposure to 0.24 g/mL of GR significantly increased both the head thrash and body bend in adult day-12 nematodes compared with controls (Fig. 5C). Therefore, GR extends the lifespan and regulates age-related properties in nematodes.

3.9. Effects of GR exposure on expression patterns of genes encoding the insulin-like signaling pathway in nematodes

In *C. elegans*, the insulin-like signaling pathway plays a key role in regulating longevity (Shen et al., 2010). After prolonged exposure, we observed that 0.24 g/mL of GR significantly increased the expression levels of *pdk-1*, *daf-18*, and *daf-16* genes, and decreased the expression levels of *daf-2*, *age-1*, *sgk-1*, and *akt-1* genes (Fig. 6A). These data indicate that the expression pattern of genes encoding the insulin-like signaling pathway was affected by GR
exposure in nematodes.

3.10. Mutation of daf-16 affects the beneficial effects of GR in nematodes

In the insulin-like signaling pathway, daf-16 gene encodes the FOXO transcription factor (Lapierre and Hansen, 2012). We found that mutation of daf-16 gene significantly suppressed the lifespan of nematodes treated with 0.24 g/mL of GR (Fig. 6B), indicating that the insulin-like signaling pathway is required for the control of the beneficial effects of GR on lifespan. Moreover, we observed that mutation of daf-16 significantly increased the induction of intestinal ROS production in nematodes (adult day-12) treated with 0.24 g/mL of GR (Fig. 6C). In addition, mutation of daf-16 gene significantly decreased the locomotion behavior of nematodes (adult day-12) treated with 0.24 g/mL of GR (data not shown). These data suggest that the insulin-like signaling pathway is involved in controlling the beneficial effects of GR on lifespan and age-related properties in nematodes.

3.11. Identification of the main chemical components in raw-processed GR

Using RP-HPLC, we identified the main chemical components in the concentrated GR solution. Based on the chromatographic fingerprint, GR contained liquiritin, isoliquiritin, glycyrrhizic acid and some other unidentified compounds (Fig. 7).

4. Discussion

GR is one of the most frequently used Chinese medicines in both western and oriental counties (Kim et al., 2006). In the present study, we first provided safety evaluation data on GR at the examined concentrations (0.12–0.24 g/mL) using C. elegans in the in vivo toxicity assay system. Our data demonstrate that prolonged exposure to GR at the examined concentrations did not induce lethality, alter body length, influence the morphology of nematodes, or affect metabolism as reflected by the pumping rate and mean defecation cycle length (Fig. 1). Moreover, prolonged exposure to GR at the examined concentrations did not alter the brood size, change the locomotion behavior, or influence the development of D-type GABAergic motor neurons controlling locomotion behavior (Fig. 2). Furthermore, prolonged exposure to GR at the examined concentrations did not cause significant induction of intestinal autofluorescence or intestinal ROS production (Fig. 3). These data suggest that prolonged exposure to the examined concentrations of GR is relatively safe in nematodes. One of the important mechanisms involved in the safety of GR is that it did not induce severe oxidative stress in targeted organs in the nematodes.

Previous studies have indicated the possible protective effects of GR on organisms. For example, GR possess high antioxidant capacity (Wojcikowski et al., 2007). GR extract attenuated renal oxidative damage induced by peroxynitrite (Yokozawa et al., 2005). GR showed protective effects against hydroxyl-induced
DNA damage and antioxidant activity (Li et al., 2013a, 2013b, 2013c). Animal experiment showed the extract of GR exhibited protective effect on liver injury of mice induced by CCl4 (Xie et al., 2009). In *C. elegans*, our data further support the protective effects of GR on animals. Pretreatment with GR effectively suppressed the damage due to heat-stress on the lifespan of nematodes (Fig. 4A). Moreover, the data presented in the current study demonstrate that the protective effects of GR may be largely due to its ability to inhibit oxidative stress. Pretreatment with GR significantly inhibited oxidative stress induced by paraquat and suppressed the damage caused by paraquat on nematode lifespan (Fig. 4B and C).

In addition to these protective effects, our data show other beneficial effects of GR, including its ability to extend the lifespan of animals. Prolonged exposure to 0.18–0.24 g/mL of GR significantly extended the lifespan of nematodes (Fig. 5A). But Yamaguchi et al found that 50–100 μg/mL GR shortened the lifespan of fer-15 mutant (*P < 0.1*) (Yamaguchi et al., 2008). Due to the concentration ranges used in the two different lifespan tests were different, the test results there may be difference. In addition, until now, more than 300 kinds of flavonoids compounds, more than 20 species of triterpene glycoside and polysaccharides were identified in the extracts of GR (Zhang and Ye, 2009). Comparing the contents of the main chemical components in the extracts of GR from different producing areas, it was found that the producing area has obvious effects on the contents of the main chemical components of GR (Li et al., 2007; Zhang et al., 2013a, 2013b). So the pharmacological action of GR collected from different area may have a little different. Moreover, we observed that prolonged exposure to GR significantly increased locomotion behavior and decreased ROS production in adult day-12 nematodes (Fig. 5B and C). These data suggest that GR treatment can suppress oxidative stress induced by the aging process.

With regard to the molecular mechanism involved in GR extending lifespan, we hypothesize that the insulin-like signaling pathway may be important in the lifespan-extending properties of GR in nematodes. Prolonged exposure to GR significantly increased the expression levels of *pdk-1*, *daf-18*, and *daf-16*, and decreased the expression levels of *daf-2*, *age-1*, *sgk-1*, and *akt-1* genes.
Comparative toxicology studies are needed to conduct, which allows common. So far our work has only been exploratory, many com-

assessment of traditional Chinese medicine using components in GR may have protective effects in organisms. For this is the case both at the level of genetic and physiological si-

toxicity of chemicals has been under study for almost 30 years (Melstrom and Hansen, 2012). Ultimately, AKT and SGK-1 phosphorylate and inactivate the transcription factor DAF-16 and block the tran-
scription of its targeted genes (Lapierre and Hansen, 2012). We found that the extended lifespan induced by GR treatment was decreased by mutation of daf-16 (Fig. 6B). The inhibition of intestinal ROS production in adult day-12 nematodes induced by GR treatment was increased by mutation of daf-16 (data not shown). Therefore, the insulin-like signaling pathway is re-

duced locomotion behavior in adult day-12 nematodes induced by GR treatment was suppressed by mutation of daf-16 (data not shown). Therefore, the insulin-like signaling pathway is re-

The use of C. elegans as alternatives to predict the potential toxicity of chemicals has been under study for almost 30 years (Williams and Dusenbery, 1988). There is increasing evidence that this is the case both at the level of genetic and physiological si-

and at the level of actual toxicity data (Melstrom and Williams, 2007; Leung et al., 2008). But the research on the risk assessment of traditional Chinese medicine using C. elegans is not common. So far our work has only been exploratory, many com-

parative toxicity studies are needed to conduct, which allows for findings from C. elegans to be extrapolated and further con-

firmed in vertebrate systems.

Many components may be present in the concentrated GR so-

tion (Fig. 7). Previous studies have reported that some of the components in GR may have protective effects in organisms. For example, liquiritigenin can prevent acute liver injuries in rats in-

duced by acetaminophen (Kim et al., 2006), and exert protective effects against heavy metal-induced toxicity (Kim et al., 2004). Diammonium glycyrrhizinate prevented murine T-cell-mediated fulminant hepatitis in an IL-10- and IL-6-dependent manner (Feng et al., 2007). Isoliquiritigenin had neuroprotective functions against Aβ-induced neurotoxicity in cultured rat cortical neurons (Lee et al., 2012). Further investigations should identify which specific component(s) account for GR extending lifespan and suppressing oxidative stress.

5. Conclusions

GR is relatively safe and has protective effects against damage due to exogenous stresses at the examined concentrations which support the notion raised previously that GR has protective effects in organisms against stresses. Furthermore, GR is capable of ex-

tending lifespan, which may be attributed to its ability to suppress the induction of intestinal ROS and regulate the expression of genes in the insulin-like signaling pathway. Our results will be helpful in understanding the possible beneficial effects of GR and its underlying mechanism.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2015.10.008.

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ritigenin, an aglycone of liquiritin in Glycyrrhiza radix, prevents acute liver in-

juries in rats induced by acetaminophen with or without buthionine.

Fig. 7. Chemical components in GR analyzed by HPLC: (A) standards chromatogram; (B) Glycyrrhizae radix decoction chromatogram. In both chromatograms, 1 represents liquiritin; 2 represents isoliquiritin; 3 represents glycyrrhizic acid. GR, Glycyrrhizae radix.