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Acanthopanax senticosus extracts have a protective effect on *Drosophila* gut immunity

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ABSTRACT

Ethnopharmacological relevance: *Aanthopanax senticosus* (*A. senticosus*) Harms is a classical adaptogenic agent used in China. It has been applied as an analeptic aid to improve weakened physical status. However, little is known about the effects of *A. senticosus* on inflammatory disease processes.

Materials and methods: Flies fed with standard cornmeal–yeast medium were used as controls, and the treatment groups contained 10% of *A. senticosus* aqueous extracts (root or fruit) in standard medium. Survival rate was performed by feeding a vial containing five layers of filter paper hydrated with 5% sucrose solution contaminated with pathogenic or toxic compounds. Imaging of the guts was viewed under the microscope. Death cells were detected by 7-AAD staining.

Results: The *A. senticosus* extract improved the survival rate, attenuated the death of intestinal epithelial cells, promoted the expression of antimicrobial peptide genes, and decreased the formation of melanotic masses. Moreover, our results indicated that the protective effect of fruit is much higher than that of root extracts.

Conclusions: *A. senticosus* extracts have a protective effect on *Drosophila* gut immunity and stress response, and may contribute to the prevention of inflammatory diseases induced by pathogenic and toxic compounds.

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1. Introduction

The intestinal epithelium, which functions as a protective barrier, is frequently exposed to multiple environmental microbes and toxic compounds. This barrier protects the host organism against invasion and systemic dissemination of both pathogenic and commensal microorganisms (Daneman and Rescigno, 2009). *Drosophila melanogaster* is a well-established model organism for the study of development and diseases. The relatively simple tissue organization, sophisticated genetic techniques, and conservation of regulatory pathways are some advantages of using *Drosophila* as a model system (Apidianakis and Rahme, 2011). The gut immunity of *Drosophila* is a complex system involving the production of reactive oxygen species (ROS) and antimicrobial peptides (AMPs). Intestinal redox balancing through the dynamic generation and elimination of ROS plays a complementary role in the intestinal immune response (Ha et al., 2005a, 2005b). In addition, local production of AMPs also contributes to inducible defense mechanisms involved in gut immunity. These two protective mechanisms are critical for keeping the commensal and non-pathogenic bacteria (Ryu et al., 2006, 2008). Recent reports demonstrated that ingestion of the Gram-negative bacterium, *Erwinia carotovora carotovora* 15 (*Ecc*15), strongly stimulates intestinal stem cell division, promoting rapid turnover of the gut epithelium (Buchon et al., 2009a, 2009b). Furthermore, some toxic compounds, such as sodium dodecyl sulfate (SDS), H_2O_2, and NaCl, affect gut homeostasis by inducing a stress response that can cause damage and apoptosis of epithelial cells (Buchon et al., 2009a, 2009b; Seisenbacher et al., 2011).

Acanthopanax senticosus (Rupr. et Maxim.) (*A. senticosus*) Harms is a plant with clinical properties that belongs to the Araliaceae family, which is also termed Siberian ginseng (Davydov and Krikorian, 2000). The major components of the *A. senticosus* aqueous extracts, including chlorogenic acid, eleutheroside B, eleutheroside B1, eleutheroside E, and isofraxidin (Takahashi et al., 2010). Several components of *A. senticosus* have been widely used for the treatment of various diseases, such as psychophysical stress, fatigue, allergic conditions and cancer, in China, Russia, Korea and Japan (Fujikawa et al., 1996; Deyama et al., 2001;

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Yi et al., 2002; Jung et al., 2003). In the last decade, attention has become increasingly focused on utilizing *A. senticosus* as a classical adaptogenic agent (Fujikawa et al., 2005). However, little is known about the effect of *A. senticosus* extract on intestinal immunity and its mechanisms of action. On the other hand, many medicinal plant such as *Mimosa pudica* and *Andrographis paniculata* has been used in the leucoderma, ulcers, dysentery, inflammation and wound healing (Sharma et al., 2001; Sheeja et al., 2006; Vinothapooshan and Sundar, 2010; Al-Bayaty et al., 2012).

In this study, we assessed the protective effect of aqueous extracts of *A. senticosus* (roots and fruits) against gut immunity and stress response in *Drosophila*. The results revealed that aqueous extracts of the roots and fruits of *A. senticosus* had beneficial effects on intestinal inflammation. Therefore, *A. senticosus* extract may improve gut immunity following ingestion of numerous pathogenic and toxic compounds.

2. Materials and methods

2.1. Drosophila strains

D. melanogaster strains were cultured on a 12 h light/12 h dark cycle at 25 °C and 60% humidity on standard cornmeal–yeast medium. W^{1118} (Bloomington *Drosophila* Stock Center) was used as the wild-type stock for each of experiments in this study.

2.2. Aqueous extracts of A. senticosus and growth medium of Drosophila

A. senticosus were purchased from Shiyitang pharmacy of Harbin, China. The dry roots and fruits of *A. senticosus* were identified by Prof. Xiuhua Wang (College of Life Sciences, Northeast Forestry University, China). A voucher specimen was deposited at the Herbarium of College of Life Sciences, Northeast Forestry University. The chopped root and fruit (20 g) were immersed in deionized water (200 ml) overnight at 25 °C. The aqueous extraction was boiled for 3 h, and extraction process was repeated twice. Total extracts were combined and concentrated to approximately 200 ml (yield: about 5–14%). Flies fed with standard cornmeal–yeast medium were used as controls, and the treatment groups contained 10% of *A. senticosus* extracts (root or fruit) in standard medium.

2.3. Survival experiments

Micrococcus luteus (*M. luteus*) (per 1.0 L distilled water:beef extract 3.0 g, peptone 5.0 g, pH 6.8) from overnight cultures (30 °C) were recovered by centrifuging at 6000 rpm for 10 min. The supernatants were discarded and the pellets were resuspended in corresponding fresh culture (Jin et al., 2008). The *Beauveria bassiana* (*B. bassiana*) (per 1.0 L distilled water:dextrose 10 g, peptone 2.5 g, yeast extract 5 g, agar 15 g) were incubated at 25 °C for 10 days. The fungus was blended with addition of PBS and filtered through glass wool. The spores of *B. bassiana* were pelleted by centrifuging at 6000 rpm and resuspended in corresponding fresh PBS. Survival and feeding experiments were performed following the procedure described by Ha et al. (2005b). Briefly, groups of 30 adult flies (15 female and 15 male) aged 3–5 days were dehydrated for 2 h without food and then transferred into a vial containing five layers of filter paper hydrated with 5% sucrose solution contaminated with *M. luteus* ($OD_{600}=180$), spores of *B. bassiana* ($OD_{600}=6$), H_2O_2 (1%, v/v), SDS (0.6%, w/v), NaCl (0.4 M) and $CuSO_4$ (6 mM). Sucrose (5%) solution with no additives served as the control for all experiments. Filter papers were changed every day.

2.4. Semi-quantitative reverse transcription-PCR

Adult flies were orally exposed to *M. luteus* ($OD_{600}=180$) or spores of *B. bassiana* ($OD_{600}=6$) and incubated at 25 °C for 6 h. Total RNA was isolated from 15–20 dissected guts (without malpighian tubules) using a TRIzol extraction (Invitrogen, Carlsbad, CA), and cDNA was generated using RevertAid Reverse Transcriptase (Fermentas). The antimicrobial peptide genes (AMPs) synthesis was subjected to 24–26 cycles of PCR amplification (94 °C for 30 s, 50 °C for 30 s and 72 °C for 40 s). All results were normalized to the *RpL*32 mRNA level for each sample. Expression levels were quantified using Image J software (NIH, Bethesda, MD), and gene expression levels were calculated as the fold change of corresponding control values. Data represent results from two independent experiments. The primer sequences are provided in Supplementary Table 1.

2.5. Imaging and 7-AAD staining

There were 8–10 guts dissected in PBS by separating the surrounding tissue in adult females. The guts were immediately viewed under the microscope. There were 8–12 dissected guts stained with 7-AAD (5 μg/ml in PBS; Invitrogen, Carlsbad, CA) for 30 min in a humidified chamber and then washed three times for 5 min in PBS. Guts were then fixed for 30 min in 4% paraformaldehyde in PBS at room temperature and washed for 5 min three times in PBS. DAPI was stained for 10 min. After washing with PBS, the guts were finally mounted in Vectashield fluorescent mounting medium (Vector Laboratories) and analyzed with an Axioskop 2 plus microscope (Zeiss). Data are representative of three independent experiments. The number of dead cells was quantified using Image J software (NIH, Bethesda, MD).

3. Results

3.1. A. senticosus improves the survival rate after oral infection with microorganisms

To analyze the effects of *A. senticosus* in *Drosophila* gut immunity, adult flies from each of the culture conditions were orally infected with *M. luteus* and spores of *B. bassiana*. The flies orally infected with *M. luteus* and fed standard medium group showed high mortality rates; however, significantly increased survival rates of 78.8% and 92.2% were observed for root and fruit extracts group, respectively (Fig. 1B, Supplementary Table 2). Following ingestion with spores of *B. bassiana* under the same conditions, the fruit of *A. senticosus* group exhibited high viability, while control and root extract groups revealed lower survival rates. Therefore, root extracts only had minor effects on survival (Fig. 1C). All tested flies exhibited high survival rates after oral exposure to 5% sucrose (Fig. 1A). These results indicate that *A. senticosus* can increase the survival rate following oral infection with microorganisms.

The wild-type W^{1118} adult flies from different culture conditions (standard, containing root and fruit extract medium) were orally ingested with sucrose (5%) (A), *M. luteus* ($OD_{600}=180$) (B), spores of *B. bassiana* ($OD_{600}=6$) (C). Con, standard cornmeal–yeast medium; root, standard medium containing 10% *A. senticosus* root extracts (v/v); fruit, standard medium containing 10% *A. senticosus* fruit extracts (v/v). Infections were performed with at least three replicates. *P*-values were calculated using the Student's *t*-test.

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