Identification of the protective effects of traditional medicinal plants against SDS-induced *Drosophila* gut damage

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Abstract. Traditional medicinal plants are widely used as immunomodulatory medicines that help improve health. A total of 50 different plants used for the treatment of toxicity were screened for their *in vivo* protective effects. Flies were fed a standard cornmeal-yeast medium (control group) or the standard medium containing medicinal plant extracts (experimental groups). Assessment of the survival rate was performed by feeding flies with toxic compounds. Gut epithelial cells were analyzed for cell proliferation and death by green fluorescent protein antibodies and 7-aminoactinomycin D staining under the microscope. The expression of antimicrobial peptides (AMPs) was evaluated by the quantitative polymerase chain reaction and the results revealed that after feeding the flies with toxic compounds, aqueous extracts from *Codonopsis pilosula* (Franch.) Nannf (*C. pilosula*), *Saussurea lappa* (Decne.) C.B.Clarke (*S. lappa*), *Imperata cylindrica* Beauv.var. *major* (Nees) C.E. Hubb. (*I. cylindrical* var. *major*) and *Melia toosendan* Sied. Et Zucc. (*M.toosendan*) increased the fly survival rate, reduced epithelial cell death and improved gut morphology. In addition, *C. pilosula* extracts induced the antimicrobial peptide levels (Dpt and Mtk) following treatment with sodium dodecyl sulfate (SDS). However, these extracts were not observed to increase SDS-induced cell proliferation *in vivo*. These results indicate that there are strong protective effects in extracts of *C. pilosula*, *S. lappa*, *I. cylindrical* var. *major* and *M. toosendan* on *Drosophila* intestinal cells among 50 medicinal plants.

Introduction

Immune responses to infection or injury are causes of systemic or local inflammation, respectively. Inflammation is a complex biological response leading to numerous diseases, including rheumatoid arthritis, chronic asthma, multiple sclerosis, inflammatory bowel disease and psoriasis (1). Inflammatory bowel disease and ulcerative colitis in particular are chronic debilitating diseases that affect millions of people worldwide. Furthermore, *Drosophila* melanogaster is a well-established model organism for studying various diseases, including inflammatory bowel diseases (2). Intestinal stem cells (ISCs) have been identified in *Drosophila* midgut and hindgut, which are equivalent to mammalian intestine and colon, respectively (3). In order to maintain gut homeostasis, intestinal epithelial cells turn over rapidly following damage from ingested pathogens, chemicals and toxic compounds. In the *Drosophila* midgut, cell turnover is functionally equivalent to that occurring in the mammalian small intestine. An ISC divides into a new ISC and a post-mitotic enteroblast (EB), which differentiates into an absorptive enterocyte or a secretory enteroendocrine cell (4). In addition, gut cell turnover is regulated by a balance between cell death and stem cell proliferation (5).

In the *Drosophila* gut, the immune response primarily relies on the local production of microbicidal reactive oxygen species (ROS) and the release of antimicrobial peptides (AMPs) (6). The production of ROS in the gut by the nicotinamide adenine dinucleotide phosphate oxidase Duox provides an efficient barrier against the majority of ingested microbes (7). However, the excessive accumulation of ROS can disrupt mitochondrial DNA, protein oxidation and lipid peroxidation, which results in impaired function of the mitochondria and metabolism (8). Furthermore, the local production of AMPs are important in the inducible defense mechanisms in the gut. AMPs are triggered by the Imd pathway through the recognition of Gram-negative peptidoglycan (9).

Traditional, medicinal plants are globally used and have rapidly grown in economic importance. Intrinsically active compounds are well-known for their anti-oxidant, anti-tumor, anti-viral and anti-inflammatory activities, and for improving immunity in general (10-12).

In the present study, *Drosophila* were used as a model organism in order to identify the protective effects of 50 different traditional medicinal plant extracts that are known to have curative or beneficial effects on the symptoms of various disorders in China. Investigating these medicinal plants, particularly the aqueous extracts of four species (*C. pilosula*, *S. lappa*, *I. cylindrical* var. *major* and

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M. toosendan), may help clinical researchers to improve their understanding of the complex roles of medicinal plants in gut disorders, including inflammatory bowel disease.

Materials and methods

Drosophila stocks. *Drosophila melanogaster* strains were cultured on a standard cornmeal-yeast medium at 25˚C and 60% humidity under a 12-h light/dark cycle. W^{1118} was purchased from the Bloomington *Drosophila* stock center (Bloomington, IN, USA), and esg-Gal4 UAS-green fluorescent protein (GFP) antibodies was a gift from Dr Rongwen Xi (National Institute of Biological Sciences, Beijing, China).

Aqueous extracts of traditional medicinal plants and preparation of growth media. A total of 50 different traditional medicinal plants were purchased from the Renmintongtai Pharmacy (Harbin, China). Aqueous plant extracts were obtained as previously described (11). A total of 50 types of traditional medicinal plants (20 g) were immersed in deionized water (200 ml; yield, ~5-14%) overnight at 25˚C. The aqueous extraction was boiled for 3 h, and the extraction process was repeated twice. The total extracts were mixed and concentrated to 100 ml. Flies fed a standard cornmeal-yeast medium were used as the control group. Flies fed the standard medium containing extracts of the medicinal plants served as the experimental groups. The final concentrations of the extracts ranged between 1.25 and 10% (w/v) (Table I).

Feeding experiments. The 4- to 5-day-old adult flies were used for the feeding experiments, with each vial containing 15 males and 15 females. Following a 2 h fast in an empty vial, flies were transferred into a vial with five layers of filter paper hydrated with 5% sucrose (w/v) with toxic compounds, containing 0.4 M NaCl, 0.6% SDS or 4% DSS. Filter papers were changed every day, and the number of living flies was recorded at each transfer for 6 or 8 days.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Due to their larger size, female flies were used for gut dissection. The survival and gut cell development were similar in both females and males (3). Adult females were treated with 1% SDS for 0, 4 or 16 h. In addition, the total RNA was extracted from 25-30 dissected guts (without Malpighian tubules) using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), and cDNA was synthesized via RT using M-MLV reverse transcriptase, RNase H minus and a point mutant kit (Promega Corporation, Madison, WI, USA). qPCR was performed in a total reaction volume of 20 μl with 3 μl DDW, 3 μl PCR primer, 10 μl master mix (2X) and 5 μl template cDNA. Lightcycler 480 SYBR Green I Master Mix was used (Roche Diagnostics, Basel, Switzerland). qPCR thermal cycling conditions were as follows: 95˚C for 5 min, followed by 40 cycles of 95˚C for 10 sec, 55˚C for 10 sec and 72˚C for 10 sec, and one melting curve cycle of 95˚C for 5 sec, 65˚C for 1 min and continuous 97˚C, followed by 40˚C for 10 sec. Results were normalized to the level of RpL32 mRNA in each sample from two independent experiments using LightCycler 480 software version 1.5 (Roche Diagnostics). Primer sequences are depicted in Table II.

Immunostaining. Dead cells were detected by 7-aminoactinomycin D (7-AAD; Invitrogen; Thermo Fisher Scientific, Inc.); gut imaging and staining were performed as described previously (11). Briefly, guts of adult females were dissected in cold phosphate-buffered saline (PBS), incubated in 7-AAD (5 μg/ml in PBS) for 30 min at room temperature, and washed with PBS three times. For immunostaining, dissected guts of female flies were fixed in 4% paraformaldehyde for 30 min at room temperature. Samples were blocked with 5% goat serum in PBS-Tween 20 (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) for 30 min followed by incubation with polyclonal anti-GFP antibodies synthesized in our laboratory (1:200) overnight at 4˚C. Following washing four times with PBS with Tween 20, samples were incubated with anti-rat IgG-fluorescein isothiocyanate secondary antibody (1:200; F1763; Sigma-Aldrich; Merck Millipore) for 2 h at room temperature adn subsequently stained with 4',6-diamidino-2-phenylindole (Sigma-Aldrich; Merck Millipore) for 10 min. Finally, the guts were mounted in 70% glycerol and imaged with an Axioskop 2 plus microscope (Zeiss AG, Oberkochen, Germany). All the data are representative of three independent experiments. The number of dead cells, intestinal stem cells and enteroblasts in the Drosophila gut was quantified using ImageJ software (V1.47; National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. Statistical analysis was performed using a two-tailed unpaired Student's t-test with Prism Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA). $P<0.005$ was considered to indicate a statistically significant difference. Error bars indicate the mean ± standard error of the mean.

Results

Medicinal plant extracts improve survival rates in vivo. The intestinal epithelium is susceptible to damage caused by pathogens, oxidative stress and toxic compounds. Foods containing SDS or NaCl could cause injury to the intestines and result in a melanotic phenotype in *Drosophila* (13). To screen for protective activities of traditional medicinal plants, flies were fed a standard cornmeal medium supplemented with (experimental groups) or without (control group) aqueous extracts of the medicinal plants. Adult flies from each of the culture conditions were orally treated with the inflammatory reagent SDS or NaCl. Initially, a vial containing 30 adult flies from each culture condition was treated with 0.6% SDS, and the survival rate was assessed over 6 days (Table III). The control group revealed >88% mortality, however, a number of flies in the experimental groups appeared to have an increased survival rate. Out of 50 different medicinal plant extracts, 16 species increased the survival rate by >50% compared with the control group (Fig. 1A). In addition, following treatment with 0.4 M NaCl, 18 species increased in survival rate by 50% compared with the control (Fig. 1B and Table IV).

In other experiments, four plant extracts that revealed a higher fly survival rate following treatment with SDS or NaCl, including *Codonopsis pilosula* (Franch.) Nannf (*C. pilosula*), *Saussurea lappa* (Decne.) C.B.Clarke (*S. lappa*), *Imperata cylindrica* Beauv.var.*major* (Nees) C.E.Hubb. (*I. cylindrical* var.

Table I. Fifty different traditional medicinal plants, plant parts and final concentrations (w/v) for screening in gut inflammation.

Latin name	Plant part
Taxillus chinensis (DC) Danser	Stem
Raphanus sativus L.	Seed
Acorus tatarinowii Schott	Rootstalk
Rheum officinale Baill.	Root and rootstalk
Peucedanum praeruptorum Dunn	Root
Trichosanthes kirilowii Maxim.	Fruit
Codonopsis pilosula (Franch.) Nannf	Root
Fructus liquidambaris	Fruit
Aconitum kusnezoffii Reichb.	Root
Cinnamomun cassia Presl.	Bark
Quisqualis indica L.	Fruit
Polygonum multiflorum Thunb.	Root
Stellaria dichatoma L.var.*lanceolata* Bge.	Root
Achyranthes Bidentata Bl.	Root
Saussurea lappa (Decne.) C.B.Clarke	Root
Pollen typhae	Pollen
Dianthus superbus L.	The whole
Leonurus heterophyllus Sweet	The whole
Panax notoginseng (Burk) F. H. Chen	Rootstalk
Imperata cylindrica Beauv. var. major (Nees) C. E. Hubb.	Rootstalk
Ophiopogon japonicns (Thumb.) Ker-Gawl.	Root
Allium macrostemon Bunge	Stem
Salvia miltiorrhiza Bunge	Root and rootstalk
Artemisia capillaris Thunb.	Whole plant
Aconitum carmichaeli Debx.	Root
Caesalpinina sappan L.	Heartwood
Melia toosendan Sied.Et Zucc.	Fruit
Uncaria rhynchophylla (Miq.) Jacks	Stem
Lithospermum erythrorhizon Sieb. et Zucc.	Root
Spatholobus suberectus Dunn	Stem
Stephania tetrandra S.Moore	Root
Cyathula officinalis Kuan	Root
Pyrrosia lingua (Thunb.) Farwell	Leaf
Alpinia katsumadai Hayata	Seed
Dalbergia odorifera T.chen	Trunk and root
Carthamus tinctorius L.	Flower
Lilium brownii var.*viridulum* Baker.	Leaf
Ligusticum chuanxiong Hort.	Rootstalk
Cyperus rotundus L.	Rootstalk
Pbyporus umbellatus (pers.) Fries	Sclerotium
Chrysanthemum monfolium Ramat.	Flower
Sophora flavescens Ait	Root
Curcuma phaeocaulis Valeton	Rootstalk
Cynanchum glaucescens (Decne.) Hand.-Mazz	Rootand rootstalk
Curcuma aromatica Salisb.	Root
Acanthopanax gracilistylus W.W.smith	Bark
Drynaria fortunei (Kunze) J.Sm	Rootstalk
Lygodium japonicum (Thunb) Sw.	Whole plant
Sanguisorba officinalis L.	Root and rootstalk
Stemona japonica (Blume) Miq.	Root

Sophora flavescens and *Stemona japonica*, 1.25%; *Stephania tetrandra* and *cyathula officinalis*, 2.5%; *cinnamomun cassia*, 5%; other types of medicinal plant, 10% (w/v).

Table II. Primer sequences used for polymerase chain reaction analyses.

Target gene	Forward (5' to 3')	Reverse (5' to 3')
Dpt	ATGCAGTTCACCATTGCCGTC	TCCAGCTCGGTTCTGAGTTG
Mtk	GCATCAATCAATTCCCGCCACC	CGGCCTCGTATCGAAAATGGG
AttA	AGGTTCCTTAACCTCCAATC	CATGACCAGCATTGTTGTAG
CecC	GATGAGCCTTTAATGTCC	TGTAAGCTAGTTTATTTCTA
Dro3	TCCACGCTGCAGAGCAC	CTAATGGAGGCCAACACTGTT
Dfn	CGCTTTTGCTCTGCTTGCTTGC	TAGGTCGCATGTGGCTCGCTTC
rp49	AGTCGGATCGATATGCTAAGCTGT	TAACCGATGTTGGGCATCAGATACT

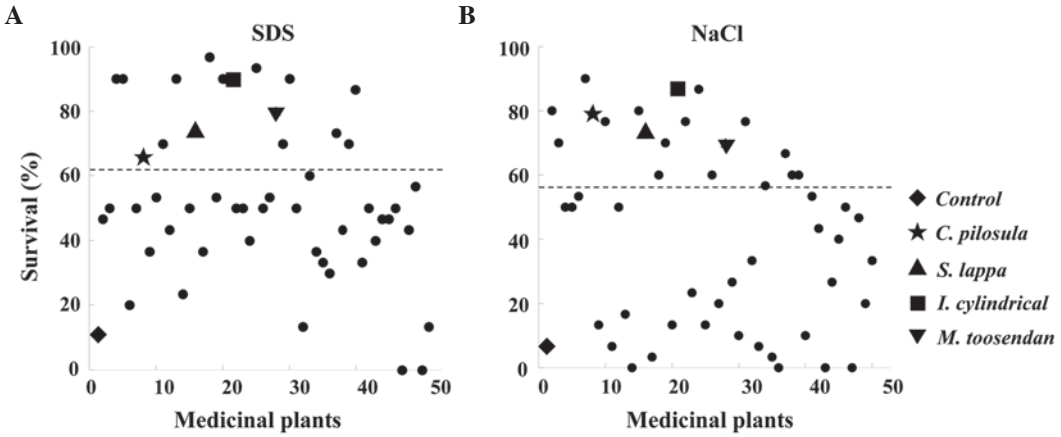

Figure 1. *In vivo* screening of the effects of anti-inflammatory reagents on the effect of 50 different traditional medicinal plant extracts. Survival rate on day 6 following treatment with (A) 0.6% SDS or (B) 0.4 M NaCl compared with the control group. The list of the 50 different traditional medicinal plants is shown in Tables III and IV. SDS, sodium dodecyl sulfate.

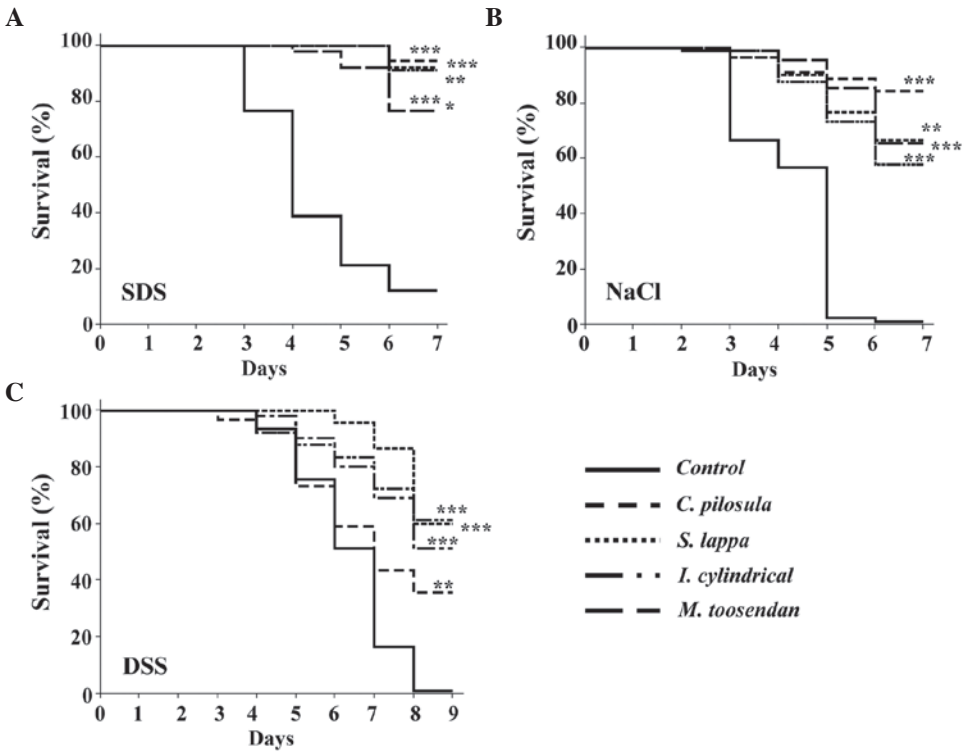

Figure 2. Survival rates of the control and experimental groups following treatment with SDS, NaCl or DSS. Adult flies cultured in standard medium (control) or supplemented with *C. pilosula*, *S. lappa*, *I. cylindrical* var. *major* and *M. toosendan* extracts were treated with 5% sucrose containing (A) 0.6% SDS, (B) 0.4 M NaCl or (C) 4% DSS at 25˚C. The survival curves were derived from three independent experiments. **P<0.005 and ***P<0.001 vs. the control group. SDS, sodium dodecyl sulfate; DSS, dextran sulfate sodium.

Table III. Survival rate of control and experimental groups that were treated with 0.6% sodium dodecyl sulfate.

Group	D0	D1	D2	D3	D4	D5	D6
Control	100.0	100.0	98.5	85.7	52.8	28.8	11.2
Taxillus chinensis (DC) Danser	100.0	100.0	96.7	96.7	96.7	73.3	46.7
Raphanus sativus L.	100.0	100.0	100.0	100.0	90.0	73.3	50.0
Acorus tatarinowii Schott	100.0	100.0	100.0	93.3	93.3	90.0	90.0
Rheum officinale Baill.	100.0	100.0	100.0	93.3	93.3	90.0	90.0
Peucedanum praeruptorum Dunn	100.0	100.0	90.0	66.7	53.3	43.3	20.0
Trichosanthes kirilowii Maxim.	100.0	100.0	100.0	90.0	80.0	66.7	50.0
Codonopsis pilosula (*Franch*.) Nannf	100.0	100.0	96.7	96.7	96.7	80.0	66.7
Fructus liquidambaris	100.0	100.0	100.0	93.3	63.3	63.3	36.7
Aconitum kusnezoffii Reichb.	100.0	100.0	100.0	93.3	76.7	76.7	53.3
Cinnamomun cassia Presl.	100.0	96.7	93.3	93.3	93.3	83.3	70.0
Quisqualis indica L.	100.0	100.0	93.3	93.3	76.7	56.7	43.3
Polygonum multiflorum Thunb.	100.0	100.0	100.0	100.0	96.7	96.7	90.0
Stellaria dichatoma L.var.*lanceolata* Bge.	100.0	93.3	73.3	56.7	40.0	30.0	23.3
Achyranthes Bidentata Bl.	100.0	96.7	96.7	96.7	90.0	70.0	50.0
Saussurea lappa (Decne.) C.B.Clarke	100.0	93.3	90.0	90.0	90.0	86.7	73.3
Pollen typhae	100.0	100.0	96.7	90.0	73.3	43.3	36.7
Dianthus superbus L.	100.0	100.0	100.0	100.0	100.0	96.7	96.7
Leonurus heterophyllus Sweet	100.0	100.0	100.0	86.7	80.0	60.0	53.3
Panax notoginseng (Burk) F. H. Chen	100.0	100.0	100.0	96.7	96.7	93.3	90.0
Imperata cylindrica Beauv. var. major (Nees) C. E.Hubb.	100.0	100.0	100.0	100.0	100.0	100.0	89.9
Ophiopogon japonicns (Thumb.) Ker-Gawl.	100.0	100.0	100.0	93.3	90.0	66.7	50.0
Allium macrostemon Bunge	100.0	100.0	96.7	93.3	86.7	63.3	50.0
Salvia miltiorrhiza Bunge	100.0	96.7	96.7	90.0	80.0	50.0	40.0
Artemisia capillaris Thunb.	100.0	96.7	96.7	96.7	93.3	93.3	93.3
Aconitum carmichaeli Debx.	100.0	86.7	83.3	83.3	76.7	73.3	50.0
Caesalpinina sappan L.	100.0	100.0	100.0	96.7	66.7	53.3	53.3
Melia toosendan Sied.Et Zucc.	100.0	96.7	93.3	93.3	93.3	93.3	80.0
Uncaria rhynchophylla (Miq.) Jacks	100.0	100.0	100.0	100.0	100.0	93.3	70.0
Lithospermum erythrorhizon Sieb. et Zucc.	100.0	100.0	100.0	96.7	96.7	96.7	90.0
Spatholobus suberectus Dunn	100.0	100.0	96.7	96.7	80.0	76.7	50.0
Stephania tetrandra S.Moore	100.0	100.0	93.3	90.0	33.3	26.7	13.3
Cyathula officinalis Kuan	100.0	100.0	96.7	96.7	90.0	86.7	60.0
Pyrrosia lingua (Thunb.) Farwell	100.0	100.0	100.0	100.0	80.0	56.7	36.7
Alpinia katsumadai Hayata	100.0	100.0	93.3	80.0	50.0	40.0	33.3
Dalbergia odorifera T.chen	100.0	100.0	100.0	96.7	66.7	46.7	30.0
Carthamus tinctorius L.	100.0	100.0	100.0	100.0	93.3	90.0	73.3
Lilium brownii var.viridulum Baker.	100.0	100.0	86.7	86.7	76.7	56.7	43.3
Ligusticum chuanxiong Hort.	100.0	100.0	100.0	96.7	96.7	96.7	70.0
Cyperus rotundus L.	100.0	100.0	100.0	100.0	86.7	86.7	86.7
Pbyporus umbellatus (pers.) Fries	100.0	100.0	100.0	96.7	66.7	46.7	33.3
Chrysanthemum monfolium Ramat.	100.0	93.3	93.3	93.3	73.3	53.3	50.0
Sophora flavescens Ait	100.0	100.0	100.0	93.3	86.7	66.7	40.0
Curcuma phaeocaulis Valeton	100.0	100.0	86.7	83.3	60.0	60.0	46.7
Cynanchum glaucescens (Decne.) Hand.-Mazz	100.0	100.0	100.0	93.3	86.7	60.0	46.7
Curcuma aromatica Salisb.	100.0	93.3	90.0	73.3	56.7	53.3	50.0
Acanthopanax gracilistylus W.W.smith	100.0	96.7	83.3	53.3	16.7	0.0	0.0
Drynaria fortunei (Kunze) J.Sm	100.0	96.7	96.7	93.3	63.3	53.3	43.3
Lygodium japonicum (Thunb) Sw.	100.0	100.0	100.0	93.3	83.3	60.0	56.7
Sanguisorba officinalis L.	100.0	100.0	93.3	80.0	40.0	3.3	0.0
Stemona japonica (Blume) Miq.	100.0	96.7	73.3	56.7	33.3	33.3	13.3

Table IV. Survival rate of control and experimental groups that were treated with 0.4 M NaCl.

Group	D0	D1	D2	D3	D4	D5	D6
Control	100.0	99.5	98.2	89.5	54.4	23.5	7.2
Taxillus chinensis (DC) Danser	100.0	96.7	96.7	96.7	93.3	93.3	80.0
Raphanus sativus L.	100.0	96.7	96.7	96.7	90.0	90.0	70.0
Acorus tatarinowii Schott	100.0	96.7	86.7	80.0	73.3	63.3	50.0
Rheum officinale Baill.	100.0	96.7	86.7	80.0	73.3	63.3	50.0
Peucedanum praeruptorum Dunn	100.0	96.7	86.7	83.3	83.3	63.3	53.3
Trichosanthes kirilowii Maxim.	100.0	100.0	100.0	100.0	100.0	93.3	90.0
Codonopsis pilosula (Franch.) Nannf	100.0	100.0	100.0	96.7	96.7	90.0	80.0
Fructus liquidambaris	100.0	100.0	96.7	96.7	73.3	46.7	13.3
Aconitum kusnezoffii Reichb.	100.0	96.7	96.7	93.3	86.7	86.7	76.7
Cinnamomun cassia Presl.	100.0	100.0	93.3	86.7	50.0	20.0	6.7
Quisqualis indica L.	100.0	100.0	93.3	86.7	73.3	63.3	50.0
Polygonum multiflorum Thunb.	100.0	100.0	100.0	93.3	83.3	46.7	16.7
Stellaria dichatoma L.var.*lanceolata* Bge.	100.0	90.0	50.0	10.0	0.0	0.0	0.0
Achyranthes Bidentata Bl.	100.0	100.0	96.7	96.7	90.0	86.7	80.0
Saussurea lappa (Decne.) C.B.Clarke	100.0	100.0	96.7	96.7	90.0	90.0	73.3
Pollen typhae	100.0	96.7	90.0	76.7	40.0	13.3	3.3
Dianthus superbus L.	100.0	100.0	96.7	96.7	80.0	76.7	60.0
Leonurus heterophyllus Sweet	100.0	100.0	100.0	90.0	86.7	76.7	70.0
Panax notoginseng (Burk) F. H. Chen	100.0	96.7	96.7	93.3	73.3	43.3	13.3
Imperata cylindrica Beauv. var. major (Nees) C. E. Hubb.	100.0	93.3	93.3	93.3	93.3	93.3	86.7
Ophiopogon japonicns (Thumb.) Ker-Gawl.	100.0	100.0	96.7	96.7	93.3	76.7	76.7
Allium macrostemon Bunge	100.0	100.0	100.0	90.0	73.3	50.0	23.3
Salvia miltiorrhiza Bunge	100.0	100.0	100.0	100.0	100.0	96.7	86.7
Artemisia capillaris Thunb.	100.0	100.0	96.7	83.3	80.0	43.3	13.3
Aconitum carmichaeli Debx.	100.0	100.0	93.3	93.3	86.7	86.7	60.0
Caesalpinina sappan L.	100.0	100.0	100.0	93.3	73.3	53.3	20.0
Melia toosendan Sied.Et Zucc.	100.0	96.7	86.7	86.7	83.3	70.0	70.0
Uncaria rhynchophylla (Miq.) Jacks	100.0	100.0	100.0	96.7	93.3	73.3	26.7
Lithospermum erythrorhizon Sieb. et Zucc.	100.0	96.7	93.3	83.3	66.7	23.3	10.0
Spatholobus suberectus Dunn	100.0	100.0	100.0	100.0	100.0	100.0	76.7
Stephania tetrandra S.Moore	100.0	100.0	90.0	86.7	70.0	50.0	33.3
Cyathula officinalis Kuan	100.0	100.0	96.7	76.7	56.7	26.7	6.7
Pyrrosia lingua (Thunb.) Farwell	100.0	100.0	100.0	100.0	90.0	76.7	56.7
Alpinia katsumadai Hayata	100.0	100.0	100.0	96.7	80.0	36.7	3.3
Dalbergia odorifera T.chen	100.0	100.0	100.0	100.0	50.0	16.7	0.0
Carthamus tinctorius L.	100.0	100.0	100.0	100.0	93.3	90.0	66.7
Lilium brownii var.*viridulum* Baker.	100.0	100.0	100.0	96.7	93.3	76.7	60.0
Ligusticum chuanxiong Hort.	100.0	96.7	96.7	96.7	96.7	76.7	60.0
Cyperus rotundus L.	100.0	100.0	100.0	96.7	66.7	23.3	10.0
Pbyporus umbellatus (pers.) Fries	100.0	100.0	96.7	96.7	93.3	80.0	53.3
Chrysanthemum monfolium Ramat.	100.0	100.0	100.0	100.0	96.7	86.7	43.3
Sophora flavescens Ait	100.0	100.0	93.3	80.0	36.7	0.0	0.0
Curcuma phaeocaulis Valeton	100.0	100.0	93.3	90.0	70.0	60.0	26.7
Cynanchum glaucescens (Decne.) Hand.-Mazz	100.0	96.7	93.3	80.0	73.3	50.0	40.0
Curcuma aromatica Salisb.	100.0	100.0	93.3	86.7	76.7	73.3	50.0
Acanthopanax gracilistylus W.W.smith	100.0	83.3	20.0	0.0	0.0	0.0	0.0
Drynaria fortunei (Kunze) J.Sm	100.0	96.7	86.7	86.7	73.3	66.7	46.7
Lygodium japonicum (Thunb) Sw.	100.0	100.0	93.3	90.0	63.3	30.0	20.0
Sanguisorba officinalis L.	100.0	100.0	100.0	100.0	73.3	53.3	33.3
Stemona japonica (Blume) Miq.	100.0	100.0	96.7	76.7	40.0	13.3	3.3

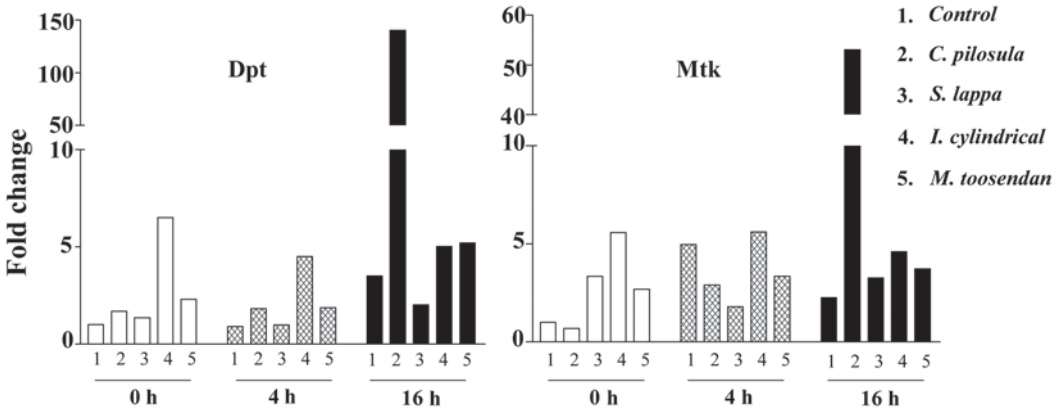

Figure 3. RNA expression levels of antimicrobial peptides (AMPs) in the adult gut. Quantitative polymerase chain reaction analysis of AMPs in adult female guts isolated from flies that were treated with sodium dodecyl sulfate for 0, 4 or 16 h. Similar expression patterns were observed in two independent experiments. Dpt, Diptericin; Mtk, Metchnikowin.

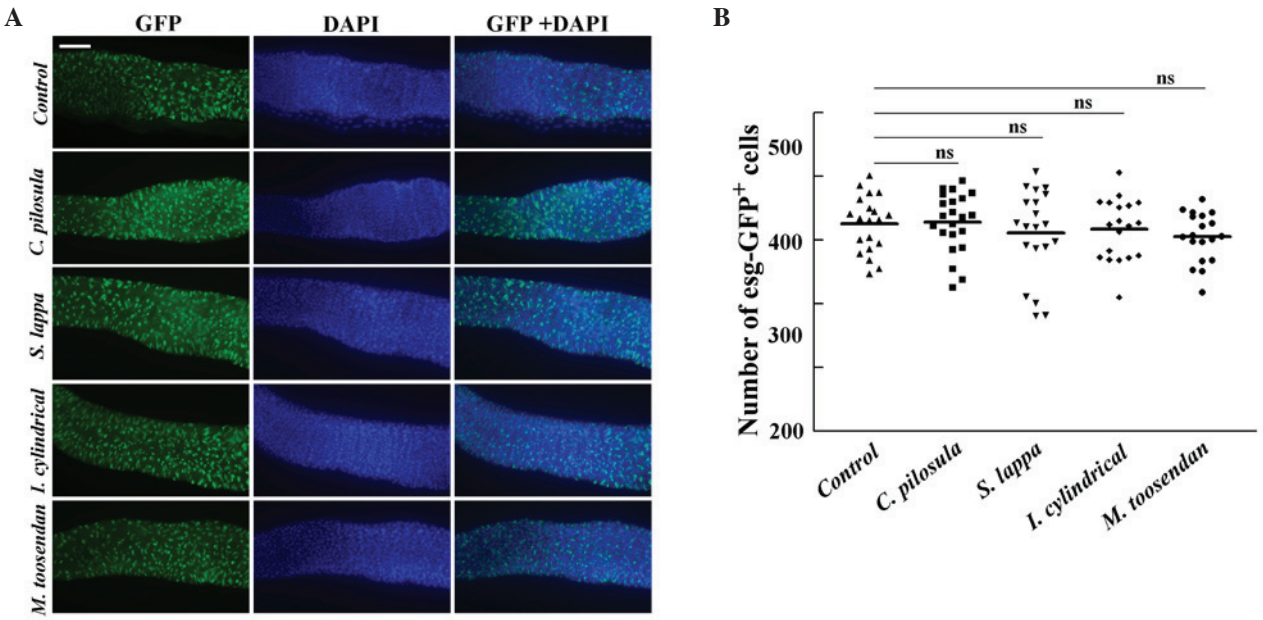

Figure 4. Sodium dodecyl sulfate (SDS) treatment induces a change in the number of intestinal stem cells and enteroblasts in the *Drosophila* gut. (A) Expression of GFP under the control of the esg-Gal4 UAS-GFP specific reporter gene in intestinal stem cells (ISCs) and enteroblasts (EBs) was induced with 0.6% SDS for 16 h. esg-GFP, ISCs and EBs (green); DAPI, nucleus (blue); GFP + DAPI, green and blue. Scale bar, 100 μm. (B) Quantification of ISCs and EBs in the posterior midguts following SDS treatment; ≥20 guts were used per group. Error bars in the graph indicate the mean ± standard error of the mean. ns, no significant difference; GFP, green fluorescent protein; DAPI, 4',6-diamidino-2-phenylindole.

major) and *Melia toosendan* Sied.Et Zucc. (*M. toosendan*), were selected for use as test extracts. Following treatment with SDS for 6 days, the survival rates of the experimental groups were 94.4 (P<0.001), 92.1 (P<0.001), 92.1 (P<0.001) and 76.6% (P<0.005), respectively, which were significantly higher compared with the survival rate of the control group (11.17%; Fig. 2A). Similarly, the four experimental groups demonstrated significantly increased survival rates [84.4 (P<0.001), 66.6 (P<0.005), 57.7 (P<0.001) and 65.5% (P<0.001), respectively] following treatment with 0.4 M NaCl (Fig. 2B). To confirm the protective effects of the four medicinal plants, another inflammatory reagent was analyzed, DSS, which interferes with the intestinal barrier function and stimulates local and systemic inflammation, causing similar tissue damage in the gut of an adult *Drosophila* (14,15). As shown in Fig. 2C, increased survival rates of 35.5, 60, 51.1 and 61.1%, respectively, were observed for extracts of these medicinal plants compared with the control group (1.1%).

These results indicate that extracts of *C. pilosula, S. lappa, I. cylindrical* var. *major* and *M. toosendan* are able to increase the *Drosophila* survival rate following exposure to toxic compounds.

AMP levels increase following medicinal plant extract treatment. The four different medicinal plants *C. pilosula, S. lappa, I. cylindrical* var. *major* and *M. toosendan* have a strong protective effect against SDS-induced gut damage, therefore, the pharmacological functions against SDS damage were analyzed. AMP-mediated defenses are capable of enhancing the stress response in adult flies and are regulated by the

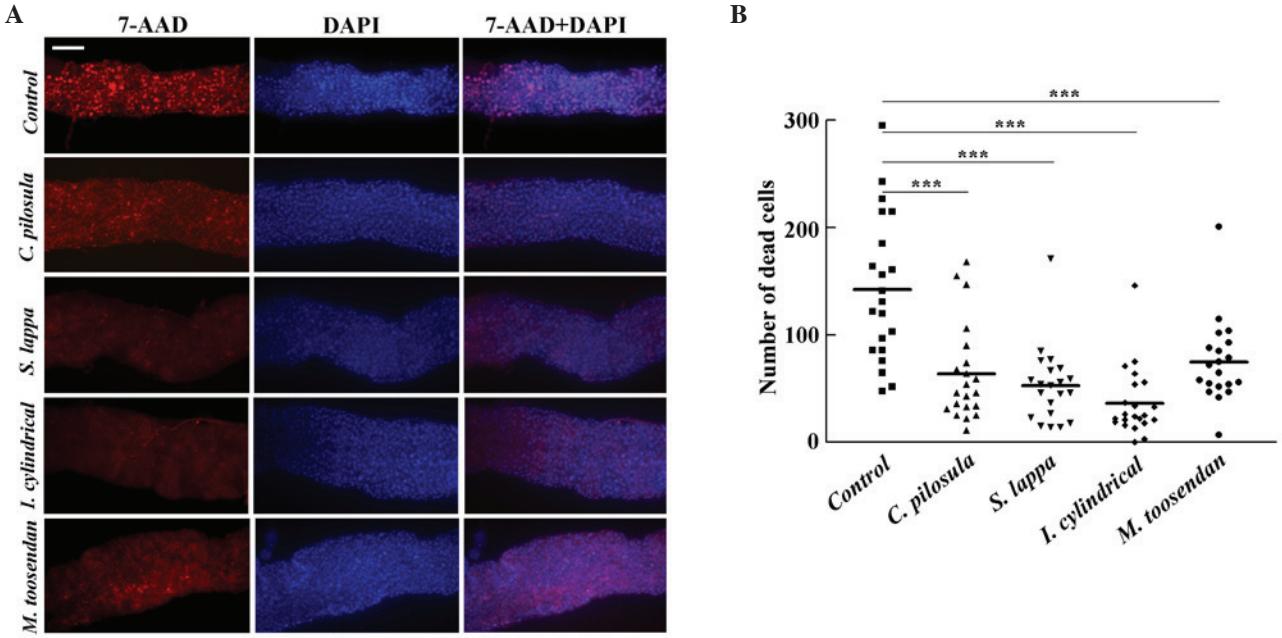

Figure 5. Protective effects of medicinal plant extracts against epithelial cell death in the *Drosophila* gut following treatment with sodium dodecyl sulfate (SDS). (A) The guts of control and experimental flies were stained with 7-AAD following treatment with 0.6% SDS for 96 h. 7-AAD, dead cells (red); DAPI, nucleus (blue); 7-AAD + DAPI, red and blue. Scale bar, 100 μm. (B) Quantification of dead cells in the anterior midgut following SDS treatment; ≥20 guts were used per group. Error bars in the graph indicate the mean ± standard error of the mean. ***P<0.001. SDS, sodium dodecyl sulfate; AAD, aminoactinomycin; DAPI, 4',6-diamidino-2-phenylindole; 7-AAD, 7-aminoactinomycin D.

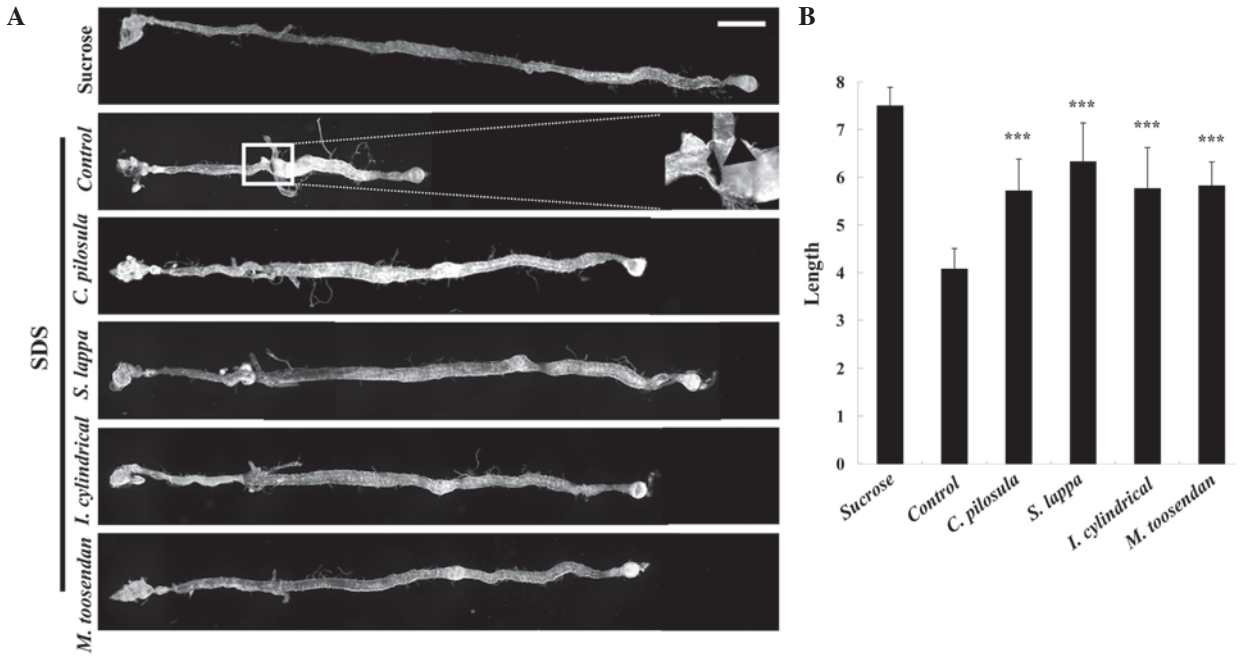

Figure 6. Protective effects of medicinal plant extracts against morphological changes in the *Drosophila* gut following treatment with SDS. (A) Nomarski images of the *Drosophila* gut following treatment with 5% sucrose (negative control group) or 0.6% SDS dissolved in 5% sucrose for 4 days. Scale bar, 500 μm. The melanotic mass is shown in the magnified image of the square box of the control group. (B) The relative lengths of guts in panel (A). Error bars represent the standard deviation. ***P<0.001 vs. the control group. SDS, sodium dodecyl sulfate.

Imd pathway (16). In order to determine whether extracts of these four medicinal plants can reduce *Drosophila* intestinal damage, AMP levels were analyzed (Dpt, Diptericin; Mtk, Metchnikowin) using qPCR. As shown in Fig. 3, slightly increased AMP levels in the experimental groups were observed compared with the controls. In addition, Dpt and Mtk RNA levels were increased in the *C. pilosula* feeding group 16 h after SDS treatment, with 40-and 23.5-fold increases, respectively, compared with the control group. The extracts of *S. lappa, I. cylindrical* var. *major* and *M. toosendan* did not significantly affect the AMP levels in the *Drosophila* gut. Furthermore, the RNA levels of other AMPs (AttA, AttacinA;

CecC, Cecropin C; Dro3, Dromycin-like peptides 3; Dfn, Defencin) were similar between groups (data not shown). These results indicate that extracts of *C. pilosula* can induce high levels of Dpt and Mtk 16 h after treatment with SDS in the *Drosophila* gut.

Medicinal plant extracts do not increase SDS-induced ISC proliferation in the midgut. Following ingestion of toxic compounds, including SDS or DSS, *Drosophila* ISCs increase their rate of proliferation in response to tissue damage (14). To analyze the protective effects of the four different medicinal plant extracts, the esg-Gal4 UAS-GFP marker (for ISCs and EBs) was used to assess adult flies following treatment with 0.6% SDS. Furthermore, the numbers of ISCs and EBs were not significantly different between groups (Fig. 4). This result indicates that these medicinal plant extracts do not induce stem cell proliferation in the *Drosophila* midgut in response to SDS.

Medicinal plant extracts are able to reduce SDS-induced cell death. In the *Drosophila* midgut, exposure to toxic compounds can increase apoptosis of epithelial cells (11). To determine whether the increased survival rate of adult flies resulted from decreased cell death in response to SDS, adult flies were treated with 0.6% SDS for 96 h. A larger number of dead epithelial cells were observed in the control group, however, flies fed with extracts of *C. Zpilosula, S. lappa, I. cylindrical* var. *major* and *M. toosendan* demonstrated significantly reduced 7-AAD signals (46.3, 38.2, 26.5 and 54.4%) compared with the control flies, respectively (P<0.001; Fig. 5). This result indicates that extracts of *C. pilosula, S. lappa, I. cylindrical* var. *major* and *M. toosendan* can increase epithelial cell viability following toxic compound treatment.

Medicinal plant extracts have protective effects against SDS-induced gut damage and morphological changes. It has previously been reported that SDS is able to induce melanotic tumors and morphological changes in the *Drosophila* gut (11). Following treatment with 0.6% SDS for 4 days, the guts of control flies appeared shorter than that of the group that was fed with sucrose. Furthermore, melanotic tumors were observed in the posterior midguts of control flies (Fig. 6A). However, the gut length of the *C. pilosula-, S. lappa-, I. cylindrical* var. *major* and *M. toosendan* extract-fed groups revealed significantly increased gut lengths compared with the control group, similar to the sucrose fed groups (P<0.001; Fig. 6A and B). In addition, no melanotic masses were observed in the *C. pilosula-, S. lappa-, I. cylindrical* var. *major-* and *M. toosendan* extract fed groups (Fig. 6A).

Discussion

Traditional medicinal plants have been effectively used with few side effects and over a long period of time (17). However, due to the large number of diverse plant species and complex multicomponent systems, the active components and pharmacological functions of numerous of these plants have not been defined. Therefore, the use of these plants as sources of novel drugs must still be explored.

In order to screen the protective effects of medicinal plant extracts *in vivo*, *Drosophila* were used as a model organism, and adult flies were treated with toxic compounds. Of 50 different medicinal plant extracts, 8 and 9 species significantly increased the survival rates >70% compared with the controls following treatment with SDS or NaCl, respectively (Tables III and IV). Among these extracts, however, a protective effect against SDS or NaCl was not identified. Furthermore, *P. multiflorum* Thunb., *P. notoginseng* (Burk) F. H. Chen, *L. erythrorhizon* Sieb. et Zucc. and *C. rotundus* L. protect against SDS-induced gut damage but do not increase the survival rate following NaCl treatment. This observation suggests that distinct mechanisms exist for these functions.

Medicinal plants that have broad protective effects against SDS and NaCl were selected for further investigation. Extracts of *C. pilosula, S. lappa, I. cylindrical* var. *major* and *M. toosendan* were used to examine their protective properties in the *Drosophila* intestine (Figs. 1 and 2). Furthermore, *C. pilosula* can be used to invigorate the function of the spleen, which is beneficial to the liver and has anti-tumor, anti-oxidant and antimicrobial properties (18-21). Its primary constituents include polysaccharides, saponins, sesquiterpenes, polyphenolic glycosides, alkaloids, polyacetylenes, essential oils and phytosteroids (22). *S. lappa* is a traditional herbal medicine that has been used to treat asthma, inflammation, rheumatism, coughs, tuberculosis and numerous other diseases (23). It contains numerous sesquiterpene lactones, flavonoids, lignans, phenyl propanoids, alkaloids, triterpenes and phytosterols (24). *I. cylindrical* var. *major* is commonly used as a diuretic and is an anti-inflammatory agent in traditional Chinese medicine (25) that exhibits diverse pharmacological activities, including cytotoxicity, neuroprotection and vasodilation (26). However, its active compounds remain unclear. Furthermore, *M. toosendan* has been widely used for the treatment of malaria, stomach aches caused by round worms or as an anti-helminthic, antiseptic and anti-inflammatory analgesic. In addition, it primarily contains limonoids, toosen-danin and triterpenoid derivatives (27).

Although the medicinal plants used in the present study have been previously explored, the majority of the results were limited to *in vitro* studies, with only a few researchers investigating their pharmacological roles *in vivo* (28,29). To the best of our knowledge, there are no references with regard to their protective effects in gut immunity. In the present study, high survival rates were observed in the experimental groups following treatment with toxic compounds. The previous studies indicated that following ingestion of pathogenic or toxic compounds, the proliferation of ISCs increased to replace dead cells, which was required for tissue homeostasis (14). Following treatment with SDS, large numbers of 7-AAD-stained cells were detected in the control group, however, only a few dead cells were observed in the groups fed with plant extracts (Fig. 5). These plant extracts decreased epithelial cell damage and melanotic tumor formation, protected the gut morphology and significantly improved the survival rates of adult flies following toxic compound treatment. However, there were no differences between groups with regard to stem cell proliferation (Fig. 4). In addition, only extracts of *C. pilosula* significantly increased AMP levels following treatment with SDS for 16 h, whereas extracts of *S. lappa, I. cylindrical* var. *major* and *M. toosendan*

were observed similar to the controls (Fig. 3). The correlation between gut microbiota and the host immune system is important in the health of an organism, and the dysregulation of this balance can lead to chronic inflammation and initiate tumor formation (30,31). The extracts of *S. lappa, I. cylindrical* var. *major* and *M. toosendan* may contribute to the basal host immune system in the *Drosophila* intestine.

In summary, the present study provides a foundation for the effective screening of a large number of pharmacological functions from traditional medicinal plant extracts. The present study demonstrated that extracts of four different traditional medicinal plants (*C. pilosula, S. lappa, I. cylindrical* var. *major* and *M. toosendan*) have protective effects against gut disorders in *Drosophila*. These results may provide a pharmacological basis for the treatment of inflammatory bowel diseases in humans.

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