Effect and mechanism of total ginsenosides repairing SDS-induced *Drosophila* enteritis model based on MAPK pathway

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Received September 28, 2022; Accepted February 22, 2023

DOI: 10.3892/etm.2023.12068

Abstract. Inflammatory bowel disease (IBD) is a chronic recurrent gastrointestinal disease that seriously endangers human and animal health. Although the etiology of IBD is complex and the pathogenesis is not well understood, studies have found that genetic predisposition, diet and intestinal flora disorders are the main risk factors for IBD. The potential biological mechanism of total ginsenosides (TGGR) in the treatment of IBD remains to be elucidated. Surgery is still the main strategy for the treatment of IBD, due to the relatively high side effects of related drugs and the easy development of drug resistance. The purpose of the present study was to evaluate the efficacy of TGGR and explore the effect of TGGR on the intestinal inflammation induced by sodium dodecyl sulfate (SDS) in *Drosophila* and to initially explain the improvement effect and mechanism of TGGR on *Drosophila* enteritis by analyzing the levels of *Drosophila*-related proteins. During the experiment, the survival rate, climb index and abdominal characteristics of the *Drosophila* was recorded. Intestinal samples of *Drosophila* were collected for analysis of intestinal melanoma. The oxidative stress related indexes of catalase, superoxide dismutase and malondialdehyde were determined by spectrophotometry. Western blotting detected the expression of signal pathway-related factors. The effects of TGGR on growth indices, tissue indices, biochemical indices, signal pathway transduction and related mechanisms of SDS-induced *Drosophila* enteritis model were studied. The results showed that TGGR could repair SDS-induced enteritis of *Drosophila* through MAPK signaling pathway, improve survival rate and climbing ability and repair intestinal damage and oxidative stress damage. The results suggested that TGGR has potential application value in the treatment of IBD and its mechanism is related to the downregulation of phosphorylated (p)-JNK/p-ERK levels, which provides a basis for drug research in the treatment of IBD.

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Abbreviations: IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; SDS, sodium dodecyl sulfate; TGGR, total ginsenosides; SOD, superoxide dismutase; MDA, malondialdehyde; CAT, catalase

Key words: TGGR, inflammatory bowel disease, oxidative stress, *Drosophila*, MAPK

Introduction

In recent years, the incidence of inflammatory bowel disease (IBD) has increased and become an important public health problem. IBD can be divided into two main pathological subtypes: Ulcerative colitis (UC) and Crohn's disease (CD). UC is a chronic non-specific colonic inflammation, which mainly affects the mucosa of the colon (1,2) UC usually starts from the distal colon, develops to the proximal and may involve the entire colon (3). The main clinical manifestations are diarrhea, abdominal pain and mucus, pus and blood in the stool (4). Yan *et al* (5) found that in preclinical models of colitis, leucine-rich repeat kinase 2 (LRRK2) deficiency ameliorated dextran sodium sulfate (DSS)-induced colitis progression, whereas the processes were aggravated by R1441C mutation. CD is a chronic granulomatous inflammation (6,7), which can affect all parts of the gastrointestinal tract, but tends to occur in the distal ileum and right colon (8). The main clinical manifestations are abdominal pain, diarrhea and intestinal obstruction (9). At present, the pathogenesis and mechanism of IBD are remain to be elucidated and a previous study showed that IBD is caused by the interaction of genetic factors, intestinal epithelial barrier damage, intestinal microbial disorders, intestinal innate immune disorders, oxidative stress injury and other factors (10). Due to the mild medicinal properties of ginseng, its active substance, total ginsenosides (TGGR) has good anti-inflammatory and antioxidant properties but the repair effect and mechanism of TGGR ·on SDS-induced enteritis model of *Drosophila melanogaster* are rarely studied (11). In order to address the global burden of challenging IBD, attention should be paid to IBD interventions and preventive measures.

The human intestine is the main organ for food digestion, absorption and metabolism. It is often affected by foreign antigens, microorganisms and other harmful substances, resulting in oxidative stress, intestinal tissue damage and continuous inflammatory response and even carcinogenesis in serious cases. Intestinal redox equilibrium plays a key role in the occurrence and progression of IBD. When the oxidative balance is broken, oxidative stress occurs, leading to oxidative damage of lipid, protein and DNA in intestinal cells, leading to apoptosis (12,13). Through the activation of REDOX sensitive signaling pathways and transcription factors, a large number of intestinal inflammatory factors and mediators are generated to induce inflammation (14). Reactive oxygen species (ROS) levels are higher in IBD intestinal inflammatory areas and ROS levels are correlated with the severity of IBD (15). Phagocyte secretion of O_2^-, H_2O_2 and OH^- in arthritic accumulation, leading to lipid peroxidation, produce the oxidation products, such as MDA and ROS which increase inflammatory white blood cells in the immune response of IBD, furthering tissue damage, which also causes superoxide dismutase (SOD) generation in places of high levels of oxidative stress. A previous study showed that excessive ROS production can cause the disorder of cytoskeleton in epithelial cells, resulting in intestinal mucosal barrier dysfunction (16). The inflammatory response, on the other hand, produces more ROS. IBD is a chronic inflammation in which a large number of white blood cells accumulate at the site of inflammation. These cells not only produce pro-inflammatory cytokines, but also produce an excess of ROS, leading to an increase in oxidative stress in the gut. Thus, the structure and function of intestinal mucosal barrier are impaired and the intestinal response ability to luminal symbiotic flora and pathogens is affected (13). In a mouse model of colitis, the content of HNE and malondialdehyde in the inflammatory mucosa of the colon was increased (17). Polysaccharide from non-medicinal parts of chrysanthemum can reduce the activities of myeloperoxidase (MPO) and NO in mice with colitis, while increasing the activity of SOD and improving inflammation in IBD mice (18). It was found that the concentration of 8-OHDG (a biological marker of DNA oxidative damage) in colonic mucosa of rats with enteritis doubled (14). All these studies suggest that IBD can intensify oxidative stress in intestinal tract, weaken the antioxidant capacity of endogenous antioxidant system and cause oxidative stress damage to biomacromolecules. Therefore, oxidative stress repair is considered to play a key role in inflammatory bowel disease and may be a new target for the development of new treatments for IBD.

At present, the clinical treatment of IBD mainly consists of drugs and surgery, but drug therapy is often accompanied by adverse reactions, which limits the use of drugs, so its effect cannot be guaranteed (19). For example, Guo and Zhang (20) found that the most common side effects of aminosalicylic acid preparation for treatment included headache, vomiting, diarrhea and skin allergic reaction. In addition, aminosalicylic acid also has toxic effects on the kidney (21). Glucocorticoids have serious side effects and long-term use can lead to infertility and growth disorders in children (22). Surgical treatment also has the risk of complications such as pelvic infection, massive bleeding and intestinal perforation and is limited by the patient's age and physical condition (23). Therefore, it is important to develop new treatments for IBD. In this context, it is significant to find and use a mild natural Chinese herbal medicine to repair IBD and this has become a hot research topic. *Panax ginseng* C.A. Mey. (Araliaceae) is a perennial herb known as the 'King of 100 grasses' and listed as the first of the 'three treasures' in northeast China (24). Ginseng, in general, displays restorative, tonic and revitalizing properties (25). A previous study found that the main functional characteristic factor of ginseng is TGGR (26). TGGR are one of the important components of ginseng for its medicinal value. A number of *in vitro* and *in vivo* studies have found that ginsenosides Rh2, Rg1 and Rg3 and other ginsenosides have anti-inflammatory and oxidative stress inhibition activities. Sun *et al* (27) found that ginsenoside Rg1 can inhibit lipopolysaccharide (LPS)-induced overactivation of rat SN microglia and inhibit the damage of midbrain dopaminergic (DAergic) neurons by inflammatory factors through glucocorticoid receptor (GR) and G protein-coupled estrogen receptor 1 (GPER1) and their interaction with insulin-like growth factor-I receptor (IGF-IR) at multiple targets. Wang *et al* (28) found that TGGR effectively regulate mitochondrial function to cope with oxidative stress by inhibiting the generation of ROS and maintaining the potential stability of mitochondrial membrane. Park *et al* (29) found that LPS-induced mitochondrial ROS inhibits cytokine release by inhibiting MAPK and NFκB pathway activation (30). However, studies on the anti-inflammatory and antioxidant effects of TGGR in inflammatory bowel disease are rarely reported.

In ginseng, its active substance, TGGR, has good anti-inflammatory and antioxidant properties although the repair effect and mechanism of TGGR on SDS-induced *Drosophila* enteritis model of *Drosophila melanogaster* are rarely studied, therefore, based on the above research background, *Drosophila melanogaster* was taken as the research object in the present study. The effects of TGGR on growth index, tissue index, biochemical index and signal pathway transduction of SDS-induced *Drosophila* enteritis model and related mechanisms were studied to understand the internal mechanism of TGGR repairing inflammatory bowel disease, providing theoretical basis for the clinical application of TGGR in inflammatory bowel disease.

Materials and methods

Establishment and treatment of SDS-induced Drosophila enteritis model. The TGGR used in was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (UV ≥80% root extraction). The content of saponin monomer was 4% Rg1, 15-16% Re, 3-5% Rb1, 4-6% Rc, 8% Rb2 and 7% Rd. The monomer structure of TGGR is shown in Fig. 1 (30). A bottle of drosophila melanogaster (~100 females and 100 males) was presented for propagation by Professor Liu Baoyan, School of Science, Beihua University (Jilin, China). The model of intestinal inflammation in *Drosophila* was constructed using the modeling method previously described (31). First, the SDS concentration was determined by referring to the relevant literature (28). A *Drosophila* survival rate experiment was performed, followed by extraction and observation of the intestinal tract (32). After acclimating for 7 days, the adult *Drosophila* were fed with 0.5% SDS for 48 h. Second,

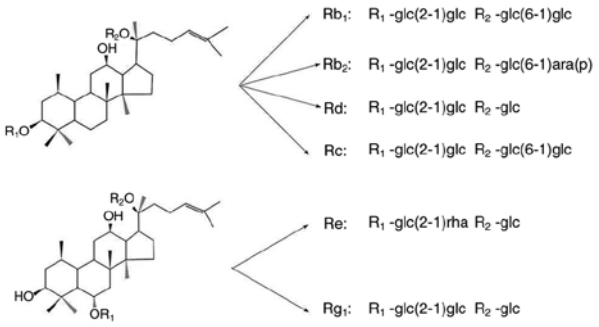

Figure 1. Structure of TGGR.

the inflammation model was verified by extracting the intestinal tract under a microscope and observing changes in its morphology. Additionally, if an intestinal melanoma was produced, the model was also be deemed to be successful. *Drosophila* grew in a constant temperature and humidity environment with the temperature of 25.0±0.5˚C and relative humidity of 50-60%. SDS induced intestinal inflammation and the flies were randomly divided into five groups with 30 (15 females and 15 males) in each group. i) Control group, ii) 0.5% SDS group, iii) 0.5% SDS +0.5% TGGR group, iv) 0.5% SDS +1% TGGR group and v) 0.5% SDS +2% TGGR group. The flies were collected and fed in common culture medium for 7 days, then transferred to empty conical flask for 4 h for starvation treatment. The corresponding solution (800 μl) was soaked into a three-layer filter paper and supplied for 48 h from day 8 to day 9. The filtrate for grouping is shown in Table I. *Drosophila* death, climbing ability and activity were recorded daily. The climbing index was detected at 48 h. When *Drosophila* were sacrificed, they were divided into two parts. One part was frozen at -80˚C ground into homogenate, and the upper clear liquid was collected for ROS, CAT, SOD, MDA detection and protein extraction. In the other part, the intestines were collected for histopathological analysis and image capture.

In the TGGR toxicity test, wild *Drosophila melanogaster* emerged for 7 days in ordinary medium were randomly divided into four groups with 30 *Drosophila* (15 females and 15 males) in each group: i) control group, ii) 0.5% TGGR group, iii) 1% TGGR group and iv) 2% TGGR group. Fruit fly deaths, climbing ability and activity were recorded daily. The present study was approved by the Laboratory Animal Ethics Committee of Beihua University [Jilin, China; approval no. beihua20210903 (1)].

Abdominal morphological indicators. After 48 h of induction, five *Drosophila* were randomly selected from each group and killed with ether. Their abdominal state was observed under an inverted microscope (CKX41SF; Olympus Corporation) and images captured with ISCapture imaging software (version 2.5.1, Tucsen).

Intestinal morphological indicators. After the *Drosophila* were treated according to the method in '*Establishment and treatment of SDS-induced Drosophila enteritis model*', 3-5 intestines of each group were removed from pre-cooled PBS and stored in sterile normal saline for observation of intestinal morphological changes and melanoma under a microscope (CKX41SF; Olympus Corporation). Images were captured with ISCapture imaging software (version 2.5.1, Tucsen).

Survival rates. The control group and the experimental group were randomly collected after seven days of emergence and 30 *Drosophila* were anesthetized with ether in each tube, with 15 males and 15 females. Newly emerging *Drosophila* was collected and fed in ordinary medium for 7 days, then transferred to empty conical flask for starvation treatment for 4 h. The corresponding solution (800 μl) required for the experiment was infiltrated into the three-layer filter paper and was continuously supplied for 48 h from day 8 to day 9. The survival numbers of *Drosophila* were recorded at 0, 12, 24, 36 and 48 h and the experiment was repeated three times.

Climbing ability. After 48 h of induction, the *Drosophila* were anesthetized with ether and placed into the track of the climbing kit. A total of three *Drosophila* were placed into each track and sealed. After all the *Drosophila* regained consciousness and adapted for at ≥20 min, the box was gently shaken to place all *Drosophila* at the bottom of the track. *Drosophila* climbed up along the inner wall because of negative taxis and the climbing distance can be measured according to the photos taken after 3 sec. Each experiment was repeated three times.

Spectrophotometric determination of SOD, MDA and CAT. After 48 h of induction, *Drosophila* were collected and frozen at -80˚C for 0.5 h, then ground into tissue homogenate with pre-cooled sterile normal saline in ice bath and centrifuged at 5,000 x g for 10 min at 4˚C. After supernatant fluid was taken, each group was evenly divided into six parts, five of which were determined by the corresponding kit for total SOD/CAT/MDA activity and the other one was determined by BCA method for protein concentration.

Western blotting. The *Drosophila* were ground and 19 μl lysate (RIPA:Protease inhibitor:phosphatase inhibitor 100:1:1; Beyotime Institute of Biotechnology) was added to 1 mg tissue. After being homogenized in a homogenizer, the *Drosophila* were centrifuged at 12,000 x g at 4˚C for 5 min. Total protein was isolated from the supernatant fluid. Protein concentration was determined by BCA Protein assay kit (Beyotime Institute of Biotechnology, Enzyme marker, Sunrise, TECAN). Each sample was added with loading buffer at a ratio of 1:5 and denatured at 100˚C for 8 min before being loaded and separated by electrophoresis with 5% SDS-PAGE (30 μg protein loaded per lane). The PVDF (Beyotime Institute of Biotechnology) membrane was activated in methanol for 1 min and the PVDF membrane and filter paper were soaked in the transport solution and marked. Then, they were assembled into a 'sandwich' in the sequence of sponge-filter paper-gel-PVDF membrane-filter paper-sponge and placed in the membrane transactor for transport. The PVDF membrane was removed and placed on a shaker in 3 ml sealing solution for 60 min, then washed with TBST (0.1% Tween-20) for 3 time/min, room temperature. The blocking procedure was 15 min at room temperature (Western Blocking Buffer; Beyotime Institute of Biotechnology). Then diluted GAPDH (Beyotime Institute of Biotechnology; 1:500; cat. no. AG019), JNK (Beyotime Institute of Biotechnology;

Table I. Establishment and treatment of SDS-induced *Drosophila* enteritis model.

	Control	0.5% SDS	0.5% SDS + 0.5% TGGR	0.5% SDS + 1% TGGR	0.5% SDS + 2% TGGR
NaOH	9 mg	9 mg	9 mg	9 mg	9 mg
NaH_2PO_4	69 mg	69 mg	69 mg	69 mg	69 mg
SDS	/	50 mg	50 mg	50 mg	50 mg
Sucrose	500 mg	500 mg	500 mg	500 mg	500 mg
ddH_2O	8 ml	8 ml	8 ml	8 ml	8 ml
TGGR	/	/	50 mg	100 mg	200 mg

SDS, sodium dodecyl sulfate; TGGR, total ginsenosides.

1:1,000; cat. no. AJ518), phosphorylated (p)-JNK (Beyotime Institute of Biotechnology; 1:1,000; cat. no. AF1762), ERK (Beyotime Institute of Biotechnology; 1:1,000; cat. no. AF1315) and p-ERK (Beyotime Institute of Biotechnology; 1:1,000; cat. no. AF1891) primary antibodies were added and incubated at 4˚C overnight. After washing with TBST for 3 times/10min, diluted secondary antibody was added (Beyotime Institute of Biotechnology; HRP-labeled Goat Anti-Mouse IgG(H+L), 1:1,000; cat. no. A0192; HRP-labeled Goat Anti-Rabbit IgG(H+L), 1:1,000; cat. no. A0208). After shaking at room temperature for 1 h, washing with TBST for 3 times/10 min. Chemiluminescence (ECL) exposure for color rendering (Syngene G; cat. no. GLC-01809; Syngene), ImageJ (v1.48; National Institutes of Health) was used for analysis.

Statistical analysis. Statistical analysis was performed by Prism software and differences between groups were analyzed by one-way ANOVA(Tukey). Data processing and statistical analysis were performed using GraphPad Prism 6 (Dotmatics).

Results

TGGR inhibits SDS-induced Drosophila enteritis. When *Drosophila* ingest harmful substances, the intestinal epidermis is the first line of defense against external infection. The protective effect of the intestinal barrier of *Drosophila* depends on the integrity of the intestine. Once the integrity of the intestine is damaged, it will cause intestinal diseases such as inflammation. Studies have found that after consuming SDS, DSS and other inflammatory factors in *Drosophila*, intestinal microbes and intestinal cells are destroyed and the intestinal environment is disturbed, thus inducing inflammation in enteritis (33). In order to explore the potential effect of TGGR on *Drosophila* enteritis, The present study caused intestinal epidermis damage by feeding 0.5% SDS to 7 day old adult Drosophila for 48 h. Compared with the control group, the survival rate of *Drosophila* in SDS group decreased. Compared with the SDS group, the survival rate of *Drosophila* treated with TGGR was significantly improved and was dose-dependent (Fig. 2A). At the same time, compared with the control group, the climbing index of the SDS group was significantly decreased, while the climbing index of the SDS + TGGR group was significantly increased (Fig. 2B), the *Drosophila* were less active due to abdominal damage. As can be seen from Fig. 2C, compared with the control group, the body color of 0.5% SDS group was deepened, the abdomen was swollen and exhibited severe abdominal edema to transparency. Compared with SDS group, SDS + TGGR group showed a dose-dependent improvement in abdominal hydrops and lighter body color. In addition, intestinal dissection revealed melanoma in the SDS group and no melanoma in the SDS + TGGR group (Fig. 2D). These results indicated that TGGR treatment can improve morphological, histological and growth indexes of SDS-induced *Drosophila* enteritis model.

TGGR restores enteritis by alleviating oxidative stress. In order to verify the antioxidant effect of TGGR, other related indexes of oxidative stress were detected by spectrophotometry. Compared with the control group, CAT activity in the 0.5% SDS group was significantly decreased. Compared with the 0.5% SDS group, 0.5% SDS + 2% TGGR group significantly inhibited the decrease of CAT content (Fig. 3A). CAT activity in 0.5% SDS + 0.5% TGGR group and 0.5% SDS + 1% TGGR group was significantly higher than that in control group and with the increase of TGGR supplemental level, CAT activity was linearly increased. SOD showed the same trend as CAT (Fig. 3B). Compared with the control group, MDA activity in 0.5% SDS group was significantly increased. Compared with 0.5% SDS group, MDA activity decreased linearly with the increase of TGGR supplemental level (Fig. 3C).

TGGR activates MAPK signaling pathway in vivo. As shown in Fig. 4, p-ERK expression in SDS group was significantly activated and p-ERK expression level decreased following TGGR treatment (Fig. 4A and B). Western blotting showed that p-JNK protein expression in SDS group was significantly inhibited by TGGR (Fig. 4A and C). In conclusion, TGGR significantly blocked MAPK signaling pathway activation in the SDS-induced *Drosophila* enteritis model. Consistent with the predicted results, TGGR can repair intestinal inflammation by reducing the level of oxidative stress and acting on intestinal epidermal damage and its related process is related to MAPK signaling pathway.

Discussion

In recent years, the incidence of IBD has been on the rise and has become a global disease (11). Invertebrates such as fruit

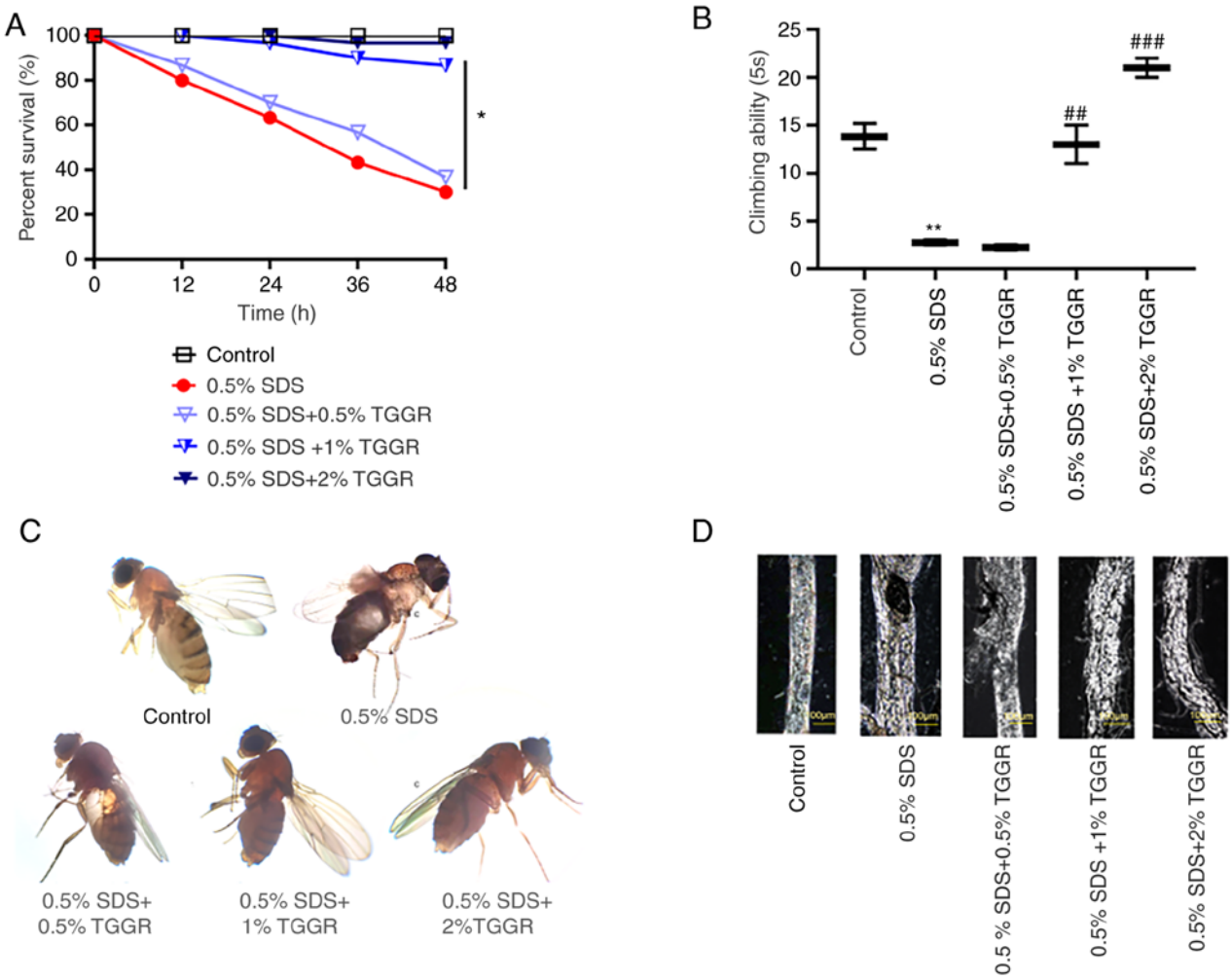

Figure 2. TGGR inhibits SDS-induced *Drosophila* enteritis (A) Percent survival. (B) Climbing ability. (C) Images of *Drosophila* abdomen. (D) Intestinal melanoma. scale bar, 100 μm, Magnification, x100, *P<0.05, **P<0.01, vs. control group; $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. 0.5% SDS only group. TGGR, total ginsenosides; SDS, sodium dodecyl sulfate.

flies have no lymphocyte B cells and T cells and can only rely on their own natural immune system to resist external invasion. Their intestinal structure is similar to that of mammals and has become a classic animal model for studying intestinal inflammatory damage and intestinal immunity. Traditional IBD drugs such as 5-aminosalicylic acid and glucocorticoids are effective but have various side effects (34). In this context, the search and use of Chinese herbs is significant for the repair of IBD. Zhou (35) found that Chinese herbs such as Codonopsis can significantly improve the intestinal inflammatory damage induced by inflammatory factors SDS and NaCI, significantly improve the survival rate, prolong the life span, inhibit the excessive proliferation of intestinal precursor cells and the apoptosis of intestinal epithelial cells, effectively maintain the intestinal morphology and promote the expression of antimicrobial peptides, which is consistent with some conclusions of the present study. The natural Chinese herbal medicine ginseng has mild medicinal properties and its active substances, TGGR, have good anti-inflammatory and antioxidant properties. Therefore, in the present study, the improvement effect and mechanism of TGGR on SDS-induced intestinal inflammation of *Drosophila* melanogaster were explored.

The present study used an SDS-induced *Drosophila* enteritis model to clarify whether TGGR can alleviate *Drosophila* enteritis symptoms. *Drosophila* treated with 0.5% SDS showed typical symptoms of IBD, such as decreased survival rate, dark abdominal body color, decreased climbing index and intestinal melanoma. TGGR can significantly improve intestinal inflammation, which showed that MDA level was significantly decreased and CAT and SOD levels are significantly increased. After pathogenic microorganisms invade the human body, the REDOX system of the body is induced to produce ROS to kill pathogens. However, the overproduction of SOD, CAT and other protective enzyme systems and the scavenging effect of antioxidants lose dynamic balance, resulting in the accumulation of ROS, oxidative damage and significant increase in the MDA content, thus aggravating the degree of membrane lipid peroxidation. These results indicated that TGGR has a good protective effect on *Drosophila* enteritis.

At present, the pathogenesis and mechanism of IBD are not completely clear and a previous study showed that IBD is caused by the interaction of genetic factors, intestinal epithelial barrier damage, intestinal microbial disorders, intestinal innate immune disorders, oxidative stress injury and other factors (36). The MAPK signaling pathway plays an important

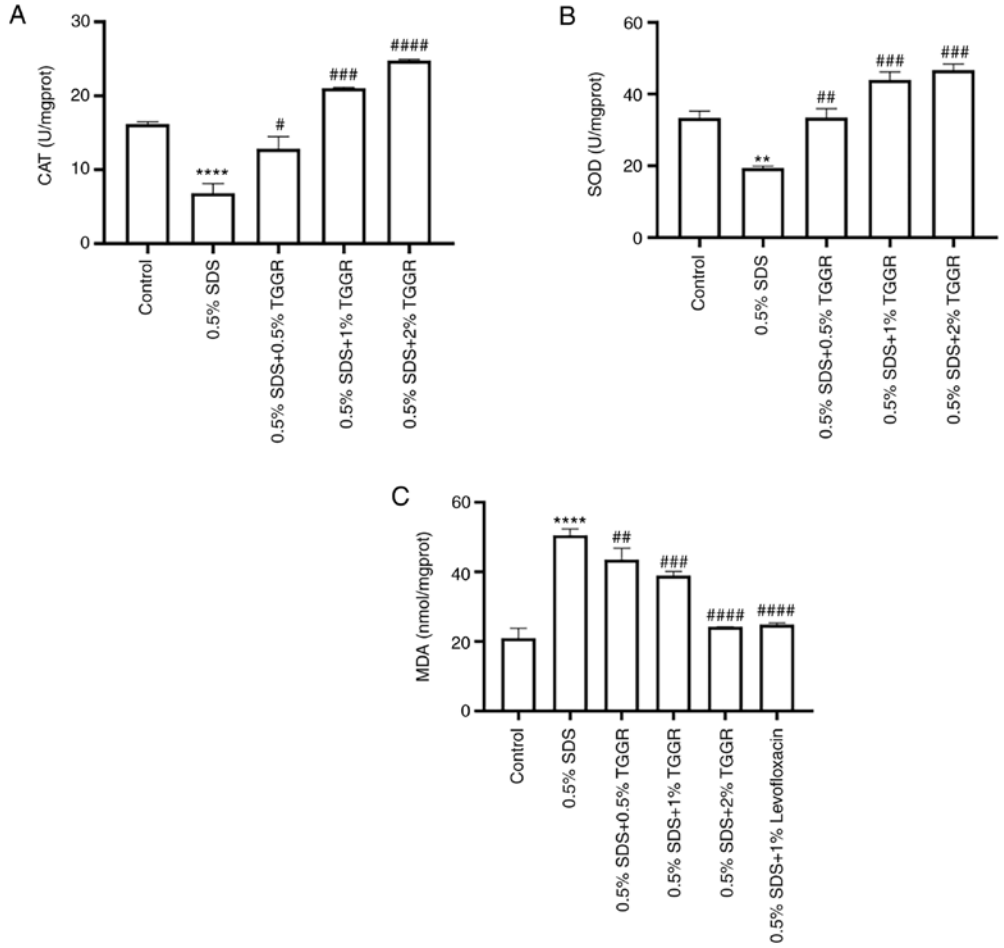

Figure 3. TGGR restores enteritis by alleviating oxidative stress. (A) Levels of oxidative stress index CAT. (B) Levels of oxidative stress index SOD. (C) Levels of oxidative stress index MDA. **P<0.01, ****P<0.0001 vs. control group; $^{\#\#}$P<0.05, $^{\#\#\#}$P<0.001, $^{\#\#\#\#}$P<0.0001 vs. 0.5% SDS group. TGGR, total ginsenosides; CAT, catalase; SOD, superoxide dismutase; MDA, malondialdehyde.

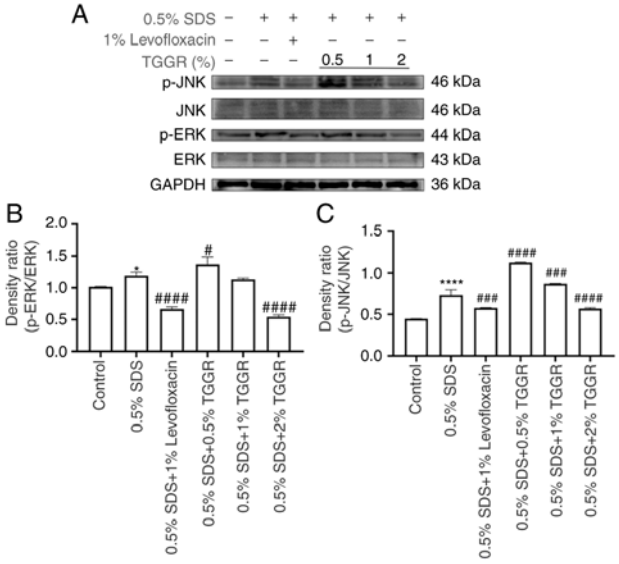

Figure 4. TGGR suppresses MAPK signaling pathway *in vivo*. (A and B) Expressions of ERK and p-ERK among drosophila proteins were examined by western blot and quantitative data was presented. *P<0.05 vs control only group; $^{\#}$P<0.05, $^{\#\#\#\#}$P<0.0001 vs 0.5% SDS only group. (A and C) Expressions of JNK and p-JNK among *Drosophila* proteins were examined by western blotting and quantitative data was presented. ****P<0.0001 vs. control only group; $^{\#\#\#}$P<0.001, $^{\#\#\#\#}$P<0.0001 vs. 0.5% SDS only group. p-, phosphorylated.

role in the regulation of inflammatory mediators (37). The MAPK family is a group of signaling molecules in the process of signal transduction, which plays an important role in the development and the occurrence and development of diseases. The MAPK family is a conserved group of serine/threonine protein kinases with three members; Extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs) and p38 mitogen-activated protein kinases (p38s). Activation of the MAPK pathway is ultimately the final step in the intracellular phosphorylation cascade. MAPK was activated by the action of MAPK/ERK kinase (MEK or MKK) by phosphorylation of tyrosine and threonine residues. The activators of ERK 1/2 are MEK1 and MEK2, the ERK5's activator is MEK5, JNK's activators are MKK4 and MKK7 and the activators of P38MAPKs are MKK3 and MKK6. The classic MAPK cascade consists of three sequential steps of intracellular protein kinase activation: It begins with the activation of MAPK kinase kinase (MAPKKK), a serine/threonine kinase that phosphorylates and activates MAPKK, which then activates MAPK by double-phosphorylation of surrounding tyrosine and threonine. As different extracellular stimuli activate different MAPK pathways and accordingly act on a variety of different substrates, a variety of specific cellular biochemical and physiological reactions will be caused. Waetzig *et al* (38) report that ERK1/ERK2, JNK/SAPK and P38 of MAPKs signal

transduction pathway are involved in intestinal mucosal injury in inflammatory bowel disease IBD. P38MAPK(A)JNK and ERK1/ERK2 are activated during intestinal mucosal epithelial injury caused by IBD. Meanwhile, MAPK can regulate the expression of inflammatory cytokines. The present study confirmed that TGGR inhibited phosphorylation of JNK. This suggested that TGGR inhibited the expression of cytokines in inflammation by decreasing JNK (MAPK). TGGR inhibited the activation of ERK1/2 and may also downregulate the expression of inflammatory cytokines by inhibiting the activation of ERK1/2 signal. In conclusion, the present study proved that TGGR can alleviate SDS-induced *Drosophila* enteritis to some extent and the mechanism may be related to the inhibition of TGGR by abnormal expression of inflammatory factors. The experimental results showed that TGGR treatment can reduce the risk of inflammatory enteritis and has certain therapeutic effects, which can be used as a potential treatment for IBD.

Overall, TGGR relieved symptoms of IBD in SDS-stimulated *Drosophila*. The therapeutic effect of TGGR is closely related to its ability to reduce levels of related proteins in the MAPK signaling pathway. These results suggested that TGGR has a prospective advantage in the design of IBD treatment strategies.

Acknowledgements

The authors would like to thank Mr. Chengyuan He and Mr. Liu Baoyan (School of Science, Beihua University, Jilin, China), for their help and discussions about the present study.

Funding

The present study was supported by Jilin Provincial Science and Technology Development Project (grant no. 20210101032JC) and Jilin City Science and Technology Innovation Development Plan Project (grant no. 20200104086).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZZ, CW and JW analyzed and interpreted the experimental results of different groups. HS and YT performed the histological examination of the *Drosophila* and were major contributors in writing the manuscript. WC and HMS conducted the data collection and confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Laboratory Animal Ethics Committee of Beihua University [Jilin, China; approval no. beihua20210903 (1)].

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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