

Docynia delavayi fruit polysaccharids alleviate DSS-Induced ulcerative colitis via oxidative stress suppression in Drosophila model

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Abstract

This study systematically investigates the polysaccharides from *Docynia delavayi* fruits, focusing on their extraction, composition, antioxidant capacity, and protective effects against intestinal damage using a DSS-induced ulcerative colitis (UC) *Drosophila* model. Employing a combination of hot water extraction and graded alcohol precipitation, distinct polysaccharide fractions (DDP-40, DDP-60, DDP-80) were obtained and characterized. The total sugar contents of DDP-40, DDP-60, and DDP-80 were 63.1%, 75.0%, and 45.0%; reducing sugar contents were 16.7%, 19.9%, and 5.7%; uronic acid contents were 6.8%, 4.6%, and 1.5%, and protein contents were 2.5%, 1.3%, and 1.8%, respectively. The primary monosaccharides of *D. delavayi* fruits polysaccharides are galactose and arabinose. *In vitro* antioxidant assays revealed that DDP-60 showed stronger DPPH and ABTS radical scavenging, whereas DDP-40 had greater ferric reducing power. In a DSS-induced *Drosophila* model, all polysaccharide fractions significantly improved survival, mitigated gut shortening, reduced epithelial permeability, and significantly inhibited intestinal epithelial cell death and ROS accumulation. This study provided novel insights into the structure-function relationship of *D. delavayi* fruits polysaccharides and their protective roles in ameliorating intestinal damage, laying a theoretical foundation for their potential development as natural therapeutic agents against UC and related intestinal disorders.

1. Introduction

Ulcerative colitis (UC) is an intestinal disease characterized by chronic inflammatory condition of the colon and oxidative stress[1]. Worldwide, the incidence of UC is significant increasing, especially in the newly industrialized countries, contributing to an increasing burden on global health care systems and health risks[2–4]. UC patients have a lower life expectancy with a risk of colorectal cancer[5, 6]. Since there is no cure, only a few medications are available to help alleviate the symptoms of UC[7]. The existing therapeutic drugs have many side effects and a high recurrence rate, resulting in poor therapeutic effects[8, 9]. Therefore, natural products have become an important direction for the development of safe and effective therapeutic drugs[8, 10, 11].

Natural polysaccharides derived from plants have gained increasing attention due to their diverse bioactivities, including antioxidant, immunomodulatory, and mucosal barrier protective effects[12, 13]. Polysaccharides from various botanical sources have demonstrated the capacity to modulate oxidative stress, attenuate inflammatory signaling pathways, and promote epithelial repair, suggesting their promise as adjunctive or alternative therapeutic agents in UC management[13, 14]. However, the “Duo-yi-guo” fruits and its polysaccharides have not yet been systematically studied for their extraction, compositional characteristics, antioxidant capacity, or potential for intestinal protection.

The fruit of *Docynia delavayi* (Franch.) Schneid., commonly known as “Duo-yi-guo”, is a kind of ethnic medicine that is recognized for its high nutritional value and serves both culinary and medicinal functions[15, 16]. It has been used clinically for the treatment of digestive disorders in traditional Chinese medicine for a long time[17]. The polysaccharides of *D. delavayi* fruit is one of the important

material bases what exerts its pharmacological activity[16]. Based on the traditional medicinal value of *D. delavayi* fruit, this article takes its polysaccharides as the research object to conduct a pharmacological efficacy study on its treatment of UC. This gap in knowledge persists despite evidence that polysaccharide molecular structures, such as monosaccharide composition and molecular weight, critically determine their bioactivity, including their ability to activate antioxidant signaling pathways and modulate immune responses[18, 19]. Accordingly, comprehensive investigation into *D. delavayi* fruit polysaccharides is warranted to elucidate their therapeutic potential.

The present study undertake a systematic evaluation of graded polysaccharide fractions extracted from *D. delavayi* fruit. Using hot water extraction and graded ethanol precipitation to obtain distinct polysaccharide fractions (DDP-40, DDP-60, DDP-80), which have detailed physicochemical and compositional characterization. The study employs *in vitro* antioxidant assays, including DPPH, ABTS radical-scavenging, and ferric reducing power evaluations, to assess the antioxidant potential of these fractions. Furthermore, a dextran sulfate sodium (DSS)-induced *Drosophila melanogaster* intestinal injury model is utilized to comprehensively evaluate the protective effects of *D. delavayi* polysaccharides on UC.

By integrating extraction methodologies, structural analysis, and multifaceted biological evaluation, this research aims to clarify the structure-activity relationships of *D. delavayi* polysaccharides and their mechanisms of action in antioxidant defense and intestinal protection. The findings are expected to provide scientific evidence supporting the development of these natural polysaccharides as novel, safe, and efficacious agents for the prevention or adjunctive treatment of UC and related intestinal disorders.

2. Materials and methods

2.1 Materials and reagents

Fruits of *D. delavayi* were obtained from Yunnan, China. W¹¹¹⁸ *Drosophila melanogaster* was obtained from the Qidong Fangjing Biotechnology Co., Ltd. (Jiangsu, China). Trifluoroacetic (TFA), phenol and monopotassium phosphate (KH₂PO₄) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Dextran T-series standards (T1000, T500, T70, T40, T10, and T5), mannose (Man), rhamnose (Rha), glucuronic acid (GluA), galacturonic acid (GalA), glucose (Glc), galactose (Gal), xylose (Xyl), arabinose (Ara) and fucose (Fuc) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. DSS (molecular weight 36–50 kDa) was obtained from Dalian Meilun Biotechnology Co., Ltd. (Liaoning Province, China). 7-Aminoactinomycin D (7-AAD) were purchased from Abcam (Shanghai) Trading Co., Ltd. Dihydroethidium (DHE) and 2',7'-dichlorodihydrofluorescein diethyl ester (DCFH-DA) were purchased from the MedChemExpress Company (MCE, Princeton, United States). Sulfuric acid was purchased from Guangzhou Reagent Co. (Guangzhou, China). All other agents were of analytical grade.

2.2 Extraction of crude polysaccharides from *D. delavayi* fruit[20, 21]

Add deionized water to 10 kg of dried *D. delavayi* fruit. Then, heat the mixture at 80°C for 3 h to extract the polysaccharides. After filtration, deionized water was added to the filter residue, and the above extraction steps were repeated three times. The extracts from the three extractions were combined and then subjected to vacuum concentration. After concentration and centrifugation, the supernatant was collected and ethanol was added with continuous stirring. During the stirring process, the ethanol concentration was measured using an alcohol meter until the ethanol concentration in the solution reached 40%. The solution was then placed in a 4°C refrigerator and allowed to stand in a sealed environment for 24 hours. Afterward, it was centrifuged to collect the precipitate, obtaining the 40% crude polysaccharides of *D. delavayi* fruits, namely DDP-40. Take the supernatant and repeat the above operation twice. Add ethanol until reaching 60% and 80% respectively, and then centrifuge separately to obtain the precipitates, which are DDP-60 and DDP-80, respectively.

2.3 Protein removal from crude Polysaccharides

Dissolve the polysaccharides of DDP-40, DDP-40 and DDP-80 in an appropriate amount of deionized water respectively. Centrifuge at a speed of 4000 r/min for 3 min to remove the insoluble impurities and precipitates and collect the supernatant. Refer to Savage method for protein removal[22], add an organic extraction solution (chloroform: n-butanol = 5:1) with a volume ratio of 1:4 to the concentrated polysaccharide solution. Shake up and down repeatedly for 20 min to allow water and organic reagents to fully contact and mix evenly. After allowing the two phases to separate, the lower protein layer is removed and centrifuged. After centrifugation, the supernatant is poured back into the separating funnel. Repeat the above operation three times. TCA is added to a final concentration of 5% after the purified protein solution is subjected to vacuum concentration. Then, mixture should be left to stand at 4°C for 12 hours, then centrifuge to obtain the supernatant. Take a dialysis bag with a molecular weight of 1000 Da, fill it with the above polysaccharide solution that has been removed of proteins. After 48 hours of continuous water dialysis, reduce the pressure and concentrate under heating conditions in a water bath at 60°C, then freeze-dry to obtain crude polysaccharides.

2.4 Determination of polysaccharide content

2.4.1 Total sugar content

Using D-glucose as the reference substance, a standard stock solution of 3.6 mg/mL was prepared. The gradient dilution method was used to prepare a series of standard working solutions at concentrations of 1.6, 0.8, 0.4, 0.2, 0.1, and 0.05 mg/mL. Using a pipette, 60 µL of the standard solution was precisely transferred and added to a 96-well microplate. Then, 30 µL of 6% phenol and 130 µL of concentrated sulfuric acid were added successively and the reaction was carried out for 15 min. The experiment was set up with three repetitions. The absorbance value was measured at 490 nm using an enzyme analyzer and the obtained data were plotted to form a glucose standard.

Take 2 mg of *D. delavayi* fruit polysaccharides and add 2 mL of deionized water to prepare a 1 mg/mL polysaccharide solution. Measure the absorbance of the polysaccharides at 490 nm, and then

incorporate this value into the standard curve to determine the content of the polysaccharides.

2.4.2 Content of reducing sugar

Precisely measure 2.00 mL of the polysaccharide sample solution into a 5 mL colorimetric tube, add 2.00 mL of DNS reagent, and conduct a reaction in a boiling water bath for 5 min. Then, cool it to room temperature and make up the volume. Use an enzyme reader to measure the absorbance at 520 nm. Perform three independent repeated measurements for each sample. Based on the standard curve obtained from the above measurements, substitute the absorbance to obtain the content of reducing sugar.

2.4.3 Measurement of Protein Content in *D. delavayi* fruit Polysaccharides

According to the protocol of the BCA kit, 5 μ L of each concentration was added to each well of the 96-well plate after diluting the protein standard into 6 concentrations. Then, 45 μ L of PBS was added to each well, followed by 200 μ L of BCA solution. The plates were incubated at 37°C for 30 min. The absorbance was measured at 570 nm using an enzyme detector to create the protein standard curve. Add 5 μ L of a 1 mg/mL polysaccharides solution to each well of the 96-well plate. Then, add 45 μ L of PBS and 200 μ L of BCA solution (A solution : B solution = 50:1) to each well. Incubate at 37°C for 30 min. Measure the absorbance at 570 nm using an enzyme-linked spectrophotometer.

2.4.4 Uronic acid content determination

Precisely weigh the glucose glucoside standard to prepare a 1.6 mg/mL stock solution. Then dilute it to 0.8, 0.4, 0.2, 0.1 and 0.05 mg/mL standard solutions. Next, add 6 mL of concentrated sulfuric acid, shake well and place in a boiling water bath for 20 min. After cooling to room temperature, add 0.2 mL of 0.15% coumarin-anhydrous ethanol reagent. Mix well and let it stand at room temperature in the dark for 2 hours. After that, aspirate 200 μ L of the solution and place it in a 96-well plate. Measure its absorbance at 530 nm using an enzyme detector. Based on the obtained absorbance values, a standard curve was plotted. Using the same method, measure the absorbance of the 1 mg/mL polysaccharide solution at 530 nm, and substitute it into the standard curve to calculate the content of uronic acid.

2.5 Analysis of the monosaccharide components in polysaccharides[23]

Take 50 μ L of each of the standard substances of arabinose (Ara), galactose (Gal), galacturonic acid (GalA), xylose (Xyl), rhamnose (Rha), glucose (Glc), glucuronic acid (GluA), mannose (Man), fucose (Fuc) into a 5 mL centrifuge tube. Mix well, then add 450 μ L of sodium hydroxide solution (0.3 M), followed by 450 μ L of freshly prepared PMP methanol solution (0.5 M). Mix again and incubate at 70°C water bath for 30 min. After the sample has completely cooled, add 450 μ L of HCl solution (0.3M) for neutralization. Then, add an equal volume of chloroform solution for extraction twice. Remove the chloroform layer,

take the aqueous phase, and filter it through a 0.22 μm filter membrane before conducting HPLC analysis.

Precisely weigh 5.00 mg of each polysaccharide sample, add 1.80 mL of 2 mol/L chromatography-grade trifluoroacetic acid solution and hydrolyze it in an oil bath at 120°C for 4 hours. After cooling to room temperature, 4 mL of methanol was added for deacidification treatment. The mixture was concentrated to dryness using a rotary evaporator and this process was repeated three times for deacidification. The hydrolyzed residue was re-solubilized with 1 mL of ultrapure water. After centrifugation for 10 min, the supernatant was collected and stored for later use. Add an equal volume of 0.3 mol/L NaOH solution to 300 μL of the hydrolyzed solution, then inject 300 μL of freshly prepared PMP-methanol solution (0.5 mol/L). After vortex mixing, conduct a 70°C constant temperature reaction for 30 min. After the reaction is terminated, 300 μL of 0.3 mol/L HCl is added for neutralization and the pH is adjusted to neutral. Perform liquid-liquid extraction with an equal volume of chloroform twice. Collect the aqueous phase, filter it through a 0.22 μm microporous membrane, and immediately conduct HPLC analysis.

2.6 The molecular weight analysis of *D. delavayi* fruit Polysaccharides[21]

The molecular weight of *D. delavayi* fruit polysaccharides were investigated via high-performance gel permeation chromatography (HPGPC), which was performed on a high-performance liquid chromatography (HPLC) system coupled with a TSK-GEL G-3000 PWXL gel column (TOSOH Bioscience, Yamaguchi, Japan) and an RID-10A refractive index detector (Shimadzu, Japan). A 0.02 M KH_2PO_4 solution was used as the mobile phase at a flow rate of 0.5 mL/min. T-series dextran standards of different molecular weights (T1000, T500, T70, T40, T10, and T5) were examined in turn to plot the calibration curve based on the retention time and the logarithm of the corresponding molecular weights.

2.7 Determination of antioxidant activity

2.7.1 DPPH free radical scavenging activity assay[24]

Precisely weigh 8.20 mg of the DPPH standard substance, dissolve it in 30 mL of ethanol (95%, v/v) and then add pure water to make the volume reach 100 mL to obtain a 0.2 mmol/L DPPH solution. Add 100 μL of DPPH solution and 100 μL of a series of gradient concentration of polysaccharide samples to the 96-well enzyme-linked plate. The absorbance measured is recorded as A_1 . 100 μL of ethanol aqueous solution was added to 100 μL of polysaccharide solution and 100 μL of DPPH solution respectively. The absorbance values measured were labeled as A_2 and A_0 , respectively. 100 μL of the DPPH solution was mixed with 100 μL of ascorbic acid solution (0.1–1.0 mg/mL) as the positive control group. After incubation at 25°C in the dark for 30 min, the absorbances were measured at 517 nm. The DPPH radical scavenging rates were calculated using the following formula:

$$\text{DPPH } (\%) = \left[1 - \frac{A_1 - A_2}{A_0} \right] \times 100\%$$

2.7.2 ABTS free radical scavenging activity assay[25]

Precisely weigh ABTS and $\text{K}_2\text{S}_2\text{O}_8$ separately and dissolve them in pure water to obtain 7.4 mmol/L and 2.45 mmol/L stock solutions respectively. Mix the above two solutions in equal volumes and let them stand under light-shielding conditions for 15 hours to generate ABTS^+ free radicals. Using PBS buffer solution with a pH of 7.4, dilute the ABTS free radical solution to an absorbance value of 0.7 ± 0.1 at 734 nm. In the 96-well microplate, 50 μL of the polysaccharide solution and 200 μL of the ABTS dilution solution were added successively. The absorbance measured was recorded as A_1 . Add 200 μL of pure water to 50 μL of the polysaccharide solution, and the measured absorbance was recorded as A_2 . Add 200 μL of ABTS dilution solution to 50 μL of pure water and the absorbance was recorded as A_0 . 200 μL of the ABTS diluent was added to 50 μL of ascorbic acid solution for a positive control group. After incubation at 25°C in the dark for 10 min, the absorbances were measured immediately at 734 nm. The ABTS radical scavenging rates were calculated using the following formula:

$$\text{ABTS } (\%) = \left[1 - \frac{A_1 - A_2}{A_0} \right] \times 100\%$$

2.7.3 Determination of iron ion reduction ability

Iron ions are abundance in living organisms and ubiquitously involved in the redox processes of the body, so the rate of iron ion clearance is an important indicator of antioxidant capacity[26]. Prepare 0.2 mol/L Na_2HPO_4 and NaH_2PO_4 stock solutions separately and mix them in a 4:6 (v/v) ratio to obtain a phosphate buffered saline (PBS) with a pH of 6.6 ± 0.1 . In a 15 mL centrifuge tube, add in sequence: 2.5 mL of PBS buffer (0.2 mol/L), 2.5 mL of 1% $\text{K}_3[\text{Fe}(\text{CN})_6]$ solution and a series of polysaccharide samples with concentrations ranging from 0.05 to 1.6 mg/mL. Mix well by vortexing and incubate at 50°C in a constant temperature water bath for 20 min. Immediately add 2.5 mL of 10% trichloroacetic acid to terminate the reaction. After centrifuging the reaction solution for 10 min, 2.0 mL of the supernatant was taken and mixed with 2.0 mL of pure water. After being placed in the dark for 10 min, the absorbance value was measured at 700 nm. The positive control used L-ascorbic acid (0.1-1.0 mg/mL). The same volume of pure water was used to replace the sample solution as the blank control.

2.8 Effects of *D. delavayi* fruit Polysaccharides on UC *Drosophila* model

2.8.1 *Drosophila* strains and rearing

Ethical permission was not required to conduct experiments on *Drosophila melanogaster*. The w¹¹¹⁸ *Drosophila* were cultured at a constant temperature of 25°C, with a 12 h light/12 h dark cycle and a relative humidity of 60%.

2.8.2 Construction of UC fly model and survival rate assay

3–5-day-old w¹¹¹⁸ flies were collected and divided into several groups: a control group (NC), a model group (DSS), three DDP-40 treatment groups (0.5%, 1%, and 2% DDP-40), three DDP-60 treatment groups (0.5%, 1%, and 2% DDP-60), and three DDP-80 treatment groups (0.5%, 1%, and 2% DDP-80). Place them in empty culture tubes without food for 2 hours to induce starvation. Then, transfer the flies to culture tubes containing three pieces of filter paper soaked with 200 µL of culture solution. Establish three experimental groups: a blank group (NC) receiving 5% sucrose solution; a model group (DSS) receiving 5% sucrose solution supplemented with 3.5% DSS; and a treatment group receiving 5% sucrose solution with 3.5% DSS plus polysaccharide solutions at three different concentrations (1%, 2%, and 5%). Feed the flies by providing the soaked filter paper for 7 days, replacing it every 24 hours, and record the daily number of deaths throughout this period. Repeat each experiment three times and plot survival curves to determine the optimal polysaccharide concentration.

2.8.3 Smurf assay

The *Drosophila* model has become a leading platform for investigating mechanisms of intestinal infiltration[27, 28]. Prepare a 2.5% blue dye solution by adding brilliant blue to a 5% sucrose solution, filter using a 0.22 µm filter, and store sterile. Female, white-eyed flies (w¹¹¹⁸) were randomly allocated into five groups (NC, DSS, DDP-40, DDP-60 and DDP-80). Each group of *Drosophila* was fed 200 µL of the corresponding solution in Table 1, replacing the filter paper every 24 hours. After 7 days of feeding, take photographs under an electron microscope. Crush the *Drosophila* using PBS, and measure absorbance at 625 nm with a microplate reader to quantify the degree of blue dye infiltration in the *Drosophila*.

Table 1
Experimental groups and corresponding solutions.

Solution	Group				
	NC	DSS	DDP-40	DDP-60	DDP-80
A. 2.5% blue dye solution (containing 5% sucrose)	A	A + B	A + B	A + B	A + B
B. 3.5%DSS solution			+ 2%DDP-40	+ 2%DDP-60	+ 2%DDP-80

2.8.4 Measurement of *Drosophila* intestinal morphology[29]

Take 30 female *Drosophila* that have emerged for 3–5 days were randomly allocated into five groups (NC, DSS, DDP-40, DDP-60 and DDP-80). Place them in empty culture tubes for 2 hours of starvation, then transfer them to culture tubes containing three pieces of filter paper and add 200 µL of culture solution. After 72 hours of culture, dissect to obtain the *Drosophila* intestines, immerse in cold PBS, fix

with 4% paraformaldehyde for 20 min, rinse three times with PBS, and observe and measure intestinal changes in each experimental group under an electron microscope.

2.8.5 7-AAD staining

Divide the collected female *Drosophila* into five groups of 30 each: a control group (NC), a model group (DSS), DDP-40, DDP-60 and DDP-80 treatment groups. After starving for 2 h, flies were transferred to empty tubes containing filter paper soaked with different media. The filter paper was impregnated with 5% sucrose in the control group, with 5% sucrose and 4% DSS in the model group, and with 5% sucrose, 4% DSS plus 2% DDP-40, DDP-60 and DDP-80 in the treatment groups. Feeding 200 μ L of the corresponding solution, replacing the filter paper every 24 hours. After 4 days of feeding, dissect the *Drosophila* in a phosphate-buffered saline (PBS) solution to obtain 10–12 intestines, place in PBS, then add 7-AAD and incubate in the dark at room temperature for 30 min. Rinse the samples with PBS three times for 5 min each to remove excess dye. Then fix the tissues with 4% paraformaldehyde (pre-cooled to 4°C) for 30 min for morphological preservation. After rinsing with PBS, add DAPI and incubate in the dark for 5 min, followed by three rinses with PBS. Mount the slides using 70% glycerol, and observe and photograph under a fluorescence microscope.

2.8.6 DHE staining

The feeding, processing, and observation steps for *Drosophila* intestines are the same as in section 2.8.5, except that 7-AAD is replaced with DHE.

2.8.7 DCFH-DA staining

The feeding, processing, and observation steps for *Drosophila* intestines are the same as in section 2.8.5, except that 7-AAD is replaced with DCFH-DA.

2.9 Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 10 (GraphPad Software, San Diego, CA, USA). Image analysis was performed using ImageJ. All experiments were performed in triplicate, and the data were presented as mean $\pm$ standard deviation (SD). Comparisons between multiple groups were analyzed via one-way or two-way analysis of variance (ANOVA). A P value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1 Determination of the chemical composition of *D. delavayi* fruit polysaccharides

3.1.1 Total sugar content

A glucose standard curve was plotted (Supplementary Figure S1A), and its linear regression equation is $Y = 2.349 \cdot X + 0.04476$, $R^2 = 0.9967$. The total sugar content of DDP-40, DDP-60, and DDP-80 are calculated as 63.1%, 75.0%, and 45.0% (Table 2), respectively. The total sugar content of the DDP-80 fraction is significantly lower than that of DDP-40 and DDP-60. This phenomenon may be attributed to the molecular weight distribution characteristics of *D. delavayi* fruit polysaccharides, which are predominantly high molecular weight polysaccharides. Based on the principle of ethanol precipitation of polysaccharides, it can be inferred that low concentrations of ethanol may preferentially precipitate high molecular weight polysaccharide components. When the ethanol concentration reaches 80%, it enriches the low molecular weight polysaccharides, leading to a significant reduction in the total sugar content of DDP-80.

Table 2				
Total polysaccharides content, reducing sugar content, uronic acid content, protein content and molecular weight of DDP-40, DDP-60 and DDP-80.				
Sample	Total polysaccharides	Reducing sugar	Uronic acid	Protein
	(%)	(%)	(%)	(%)
DDP-40	63.1%	16.7%	6.8%	2.5%
DDP-60	75.0%	19.9%	4.6%	1.3%
DDP-80	45.0%	5.7%	1.5%	1.8%

3.1.2 The content of reducing sugar

The linear regression equation of the reducing sugar standard curve is $Y = 1.707 \cdot X + 0.1343$, $R^2 = 0.9992$ (Supplementary Figure S1B). The reducing sugars contents of DDP-40, DDP-60, and DDP-80 can be calculated as 16.7%, 19.9% and 5.7% (Table 2), respectively.

3.1.3 The content of uronic acid

The linear regression equation of the uronic acid standard curve is $Y = 3.650 \cdot X - 0.06679$, $R^2 = 0.9954$ (Supplementary Figure S1C). The uronic acid contents of DDP-40, DDP-60, and DDP-80 can be calculated as 6.8%, 4.6%, 1.5% (Table 2), respectively. The uronic acid content decreases as the ethanol concentration increases, likely due to its weak hydration ability, leading to precipitation in low polarity environments, such as those with low ethanol concentrations.

3.1.4 The protein content

The linear regression equation of the protein content standard curve is $Y = 0.4917 \cdot X + 0.1463$, $R^2 = 0.9920$ (Supplementary Figure S1D). The protein contents of DDP-40, DDP-60, and DDP-80 can be calculated as 2.5%, 1.3%, 1.8% (Table 2), respectively. After protein removal using the Seavage method combined with TCA, the protein content of *D. delavayi* fruit polysaccharides was found to be extremely low, indicating that the combined protein removal process effectively eliminates proteins, which could interfere with subsequent antioxidant activity assessments.

3.2 Preliminary Structural Characterization

As shown in Fig. 1, the HPGPC of DDP-40 contain two main peaks, which are located at 12.48 min and 17.07 min, respectively. DDP-60 was shown to contain one main peak at 16.39 min and a little peak at 11.90 min. DDP-80 exhibited two peaks at 13.57 min and 17.07 min. According to the calibration curve ($\text{Log Mw} = 14.83865 - 1.30621 \cdot X + 0.06122 \cdot X^2 - 0.00135 \cdot X^3$, $R^2 = 0.9994$) presented in Supplementary Figure S2, the average molecular weights of CRP20, CRP40, CRP60 and CRP80 were calculated to be 4.64-280.61 kDa, 8.54 kDa, and 4.63-103.11 kDa, respectively. The results of molecular weight distribution indicate that low concentrations of ethanol may preferentially precipitate high molecular weight polysaccharide components.

As shown in Fig. 2, the analysis results of the monosaccharide components of *D. delavayi* fruit polysaccharides showed that DDP-40 is mainly composed of mannose, galactose and arabinose, with a molar ratio of 1.7:1.3:1. DDP-60 is mainly composed of galactose and arabinose, with a molar ratio of 1.6:1. DDP-80 is mainly composed of galactose and arabinose, with a molar ratio of 1:1.12. The results of monosaccharide composition analysis indicate that graded alcohol precipitation does not alter the types of monosaccharides but does change their molar ratios.

HPLC of the monosaccharide mixture, (B-D) The HPLC chromatograms of DDP-40, DDP-60 and DDP-80 monosaccharide component are presented in sequence.

3.3 Evaluation of the antioxidant activity of *D. delavayi* fruit polysaccharides

3.3.1 Free radical scavenging activity against DPPH

Using L-ascorbic acid as the positive control, its DPPH radical scavenging rate was 99.87%. As shown in Fig. 3A, at a concentration of 1.6 mg/mL, the maximum scavenging rate of DDP-40 for DPPH free radicals was 86.43%, that of DDP-60 was 96.93%, and that of DDP-80 was 75.42%. The nonlinear analysis of DPPH radical scavenging rate was conducted using GraphPad Prism10. The results showed that the IC_{50} values of DDP-40, DDP-40, and DDP-80 were 0.2194 mg/mL, 0.1144 mg/mL, and 0.5108 mg/mL respectively. Thus, the order of DPPH scavenging ability was: DDP-60 > DDP-40 > DDP-80.

3.3.2 Free radical scavenging activity against ABTS

Furthermore, we measured the ability of the polysaccharides to scavenge ABTS free radicals. As a result shown in Fig. 3B, at a concentration of 1.6 mg/mL, the scavenging rate of ABTS free radicals by DDP-40, DDP-60, DDP-80 was 84.56%, 92.83% and 65.83%, respectively. L-ascorbic acid was used as the positive control, and its scavenging activity against ABTS was 99.87%. It can be calculated that the IC_{50} values of DDP-40, DDP-60, and DDP-80 are 0.3700 mg/mL, 0.2971 mg/mL and 0.4982 mg/mL respectively. Thus, the order of ABTS clearance ability is DDP-60 > DDP-40 > DDP-80.

3.3.3 The reducing ability of iron ions

The chelating ability of Fe^{2+} is regarded as another important indicator for evaluating the antioxidant activity of polysaccharides. The reducing ability of the polysaccharides from *Docynia delavayi* fruits towards iron ions has been depicted in Fig. 3C. At 700 nm, when the concentration was 1.6mg/mL, the total reducing abilities of DDP-40, DDP-60, and DDP-80 for Fe^{2+} were 1.160, 0.975, and 0.573 respectively, while L-ascorbic acid had a value of 1.587. Thus, the order of total reducing ability was DDP-40 > DDP-60 > DDP-80.

Based on the scavenging rates of DPPH and ABTS free radicals and the IC_{50} values of the various polysaccharide fractions, the order of scavenging ability for DPPH and ABTS free radicals is DDP-60 > DDP-40 > DDP-80, while the order of total reducing capacity is DDP-40 > DDP-60 > DDP-80.

Polysaccharides from different fractions of *D. delavayi* fruit exhibit significant antioxidant capacity, which may be closely related to their monosaccharide composition characteristics. The main monosaccharides in the three polysaccharide fractions are mannose, galactose, and arabinose, with the hydroxyl groups of galactose potentially forming hydrogen bonds with free radicals, while arabinose may exist in a furanose form, promoting electron transfer efficiency. The synergistic effect of these two components may be a significant source of antioxidant activity. DDP-60 exhibits the strongest scavenging abilities against DPPH and ABTS free radicals, likely due to its highest total sugar content and the proportion of arabinose and galactose. However, in the iron ion reduction ability experiment, DDP-40 shows superior total reduction capacity compared to DDP-60, possibly due to its higher uronic acid content, as the carboxyl groups of uronic acid specifically bind to metal ions, increasing its total reduction capacity.

3.4 Evaluation of the pharmacological activity of *D. delavayi* fruit polysaccharides on ulcerative colitis

3.4.1 The impact on the survival rate of fruit flies

Different concentrations of DDP-40, 60, and 80 (1%, 2%, and 5% respectively) were selected for the intervention experiment on fruit fly survival rate. In the model group induced by DSS, the survival rate of fruit flies decreased to only 12.5% after 7 days of feeding, which was 83.3% lower than that of the NC group, showing a significant difference. However, after 7 days of interventional feeding the fruit flies with different concentrations of DDP-40, 60, and 80 polysaccharides, their survival rate significantly increased (Fig. 4). It indicated that the polysaccharides of *Docynia delavayi* fruit can effectively alleviate the intestinal damage in fruit flies to increase their survival rate. The results showed that all the three polysaccharides mentioned above exhibited the best activity in enhancing the survival rate of fruit flies at a concentration of 2%. Therefore, in the subsequent experiments, a 2% solution of polydextran was used for the tests.

3.4.2 The influence of *D. delavayi* fruit polysaccharides on the morphology and permeability of the fruit fly's intestinal tract

Apoptosis of intestinal epithelial cells and excessive deposition of enteroblasts may lead to changes in the morphology of the intestine. Therefore, the intestinal morphology of the fruit fly's can reflect the inflammatory state of its intestine. The intestinal tracts of each group of fruit flies were observed under a microscope. As a result shown in Fig. 5A, the intestinal lengths of the other three groups all exhibited significant shortening under the action of DSS compared with the NC group. The intestinal lengths of the drug intervention groups with DDP-40, DDP-60 and DDP-80 were significantly different from those of the DSS-induced model group (Fig. 5B). Their shapes were more similar to those of the NC group. It indicated that the polysaccharides of *D. delavayi* fruit have a good protective effect on intestinal damage.

On the other hand, we also conducted the corresponding tests on the intestinal permeability of each group of fruit flies. Dextran sulfate sodium (DSS) has a high negative charge due to its sulfate groups, which are toxic to the colonic epithelium. It can cause tissue erosion and eventually damage the barrier integrity, leading to increased permeability of the colonic epithelium. The intestinal barrier function of fruit flies can be detected by observing the infection situation of fruit flies dyed with brilliant blue dye. This allows for the assessment of the integrity of the fruit fly's intestines, thereby enabling the study of the effect of polydextran on the intestinal permeability of fruit flies. As shown in Fig. 5C, the DSS-induced model group of fruit flies showed an overall blue coloration. In the NC group, the brilliant blue dye ingested only infected the mouthparts and digestive tract. The infection levels in the DDP-40, DDP-60, and DDP-80 groups were between those of the NC group and the DSS group. The infection levels of the fruit flies in the drug intervention group were all between those of the NC group and the model group.

Take 15 fruit flies and place them in a homogenizer for homogenization. After centrifugation, take the supernatant and measure the absorbance at 625 nm for each group. The absorbance measurement results showed that the absorbance of the DSS group was 114.86% higher than that of the NC group. Compared with the DSS group, the absorbance values of the DDP-40 group decreased by 39.06%, those of the DDP-60 group decreased by 39.67% and those of the DDP-80 group decreased by 29.45% (Fig. 5D). The above three types of polysaccharides significantly alleviated the intestinal leakage in DSS-induced fruit flies. From this, it can be concluded that DDP-40, DDP-60, and DDP-80 all have a significant effect on improving intestinal permeability.

3.4.3 The effect of *D. delavayi* fruit polysaccharides on the apoptosis of intestinal epithelial cells in fruit flies

7-AAD is a fluorescent derivative of actinomycin D, which specifically binds to the GC-rich regions of DNA[30, 31]. It emits red fluorescence under an excitation wavelength of 546 nm, so 7-AAD staining is used to analyze the number of apoptotic cells in intestinal epithelial cells[32]. As shown in the Fig. 6A and B the number of apoptotic cells in the intestinal epithelial cells of DSS group increased significantly by 337.87% compared to the NC group. DDP-40 decreased by 42.75% compared to the DSS group, DDP-60 decreased by 42.78% compared to the DSS group, and DDP-80 decreased by 24.22% compared to the

DSS group. This indicates that polydioscin polysaccharides can effectively reduce the number of intestinal epithelial cell deaths and protect the intestinal tract.

3.4.4 The influence of *D. delavayi* fruit polysaccharides on the ROS level in the fruit fly's intestine

Under excessive stress conditions, cells will produce a large amount of ROS, which can damage the structure of organelles and biological molecules, thereby leading to inflammatory responses. In Fig. 6A, the results of 7-AAD, DCFH-DA and DHE staining of *Drosophila* intestines showed that the fluorescence intensity of the model group induced by DSS was significantly enhanced, while the fluorescence intensity of the administration group intervened with DDP-40, DDP-60, and DDP-80 showed varying degrees of weakening. Furthermore, we conducted a statistical analysis of the number of dead cells in each group and found that the number of apoptotic drosophila epithelial cells in the DSS group increased by 337.87% compared with the NC group, the DDP-40 group decreased by 42.75% compared with the DSS group, the DDP-60 group decreased by 42.78% compared with the DSS group, and the DDP-80 group decreased by 24.22% compared with the DSS group (Fig. 6B). Therefore, DPP-40, DPP-60, DPP-80 could all effectively inhibit the apoptosis of intestinal epithelial cells caused by DSS and protect the damaged tissues.

As shown in Fig. 6C, the DHE fluorescence intensity in the DSS group was significantly higher than that in the NC group. Compared with the DSS group, the average fluorescence intensities in the DDP-40, DDP-60, and DDP-80 groups decreased by 23.22%, 23.58%, and 12.39%, respectively. Meanwhile, we used DCFH-DA to label the ROS in the intestinal tract. After being oxidized by ROS, DCFH-DA emits green fluorescence, and its intensity is positively correlated with the total ROS level. As shown in Fig. 6D, the fluorescence intensity of the NC group was lower, and the fluorescence intensity of the DSS group was significantly higher than that of the NC group. The fluorescence intensities of the DDP-40 group, DDP-60 group, and DDP-80 group were respectively 23.24%, 23.58%, and 12.39% lower than that of the DSS group. The results consistently indicated that DDP-40, DDP-60, and DDP-80 could all reduce the ROS levels in the fruit fly's intestinal tract.

The high proportions of galactose and arabinose in the monosaccharide compositions of various fractions of *D. delavayi* fruit polysaccharides have been shown in previous studies to activate the Nrf2/Keap1 pathway, enhancing the expression of antioxidant enzymes, thereby clearing ROS and alleviating oxidative stress. In the *Drosophila* experiments, DHE and DCFH-DA staining indicated that after feeding with *D. delavayi* fruit polysaccharides, the ROS levels in *Drosophila* intestines significantly decreased. This study found that *D. delavayi* fruit polysaccharides significantly inhibited apoptosis of intestinal epithelial cells and accumulation of ROS induced by DSS.

4. Conclusion

Polysaccharides from *D. delavayi* fruits were extracted using hot water, followed by graded ethanol precipitation and protein removal using the Sevag method combined with TCA. After dialysis, different fractions of *D. delavayi* fruits polysaccharides were obtained, named DDP-40, DDP-60, and DDP-80.

Compositional analysis revealed distinct sugar, protein, and uronic acid contents across fractions, indicating ethanol-dependent selectivity influenced by molecular weight, branching, and polarity. The average molecular weights of DDP-40, DDP-60, DDP-80 were calculated to be 4.64-280.61 kDa, 8.54 kDa, and 4.63-103.11 kDa, respectively. DDP-40 is primarily composed of mannose, galactose, and arabinose in a molar ratio of 1.7:1.3:1, DDP-60 consists mainly of galactose and arabinose in a molar ratio of 1.6:1, and DDP-80 contains mainly galactose and arabinose in a molar ratio of 1:1.12.

In the *Drosophila* DSS-induced intestinal inflammation model, administration of *D. delavayi* fruits polysaccharides significantly improved survival, mitigated gut shortening, reduced epithelial permeability, and attenuated both epithelial cell death and ROS accumulation. This suggests that *D. delavayi* fruits polysaccharides may alleviate intestinal inflammation in *Drosophila* through restoring the integrity of intestinal epithelial cell barriers and inhibiting intestinal oxidative damage. Although this study underscores the protective role of *D. delavayi* fruits polysaccharides against intestinal damage, limitations include the use of graded ethanol precipitation alone, which may not fully resolve the heterogeneity and complexity of polysaccharide structures. And the *Drosophila* model, which incompletely mirrors mammalian gut physiology. Despite these limitations, the research advances our understanding of *D. delavayi* fruits polysaccharides by systematically delineating their compositional profiles, antioxidant capacities, and intestinal protective effects. Notably, the differential activities among DDP-40, DDP-60, and DDP-80 fractions highlight the importance of specific monosaccharide ratios—particularly galactose and arabinose—in modulating biological function. This work provides a foundation for structure–activity relationship studies and suggests significant therapeutic potential for these polysaccharide fractions in managing oxidative stress and intestinal barrier dysfunction. Future research should focus on fine-structure characterization, expand to mammalian colitis models, and elucidate the precise molecular pathways involved, aiming to translate these findings toward clinical application or the development of novel dietary interventions.

Declarations

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Not applicable.

Clinical trial number

Not applicable.

Ethical Approval

Not applicable. Ethical permission was not required to conduct experiments on *Drosophila melanogaster*.

Consent to Participate

All the authors consented to participate.

Consent for Publication

All the authors read and approved the final manuscript.

Authors contributions statement

Yanhong Ma: Writing – original draft, Validation, Project administration, Funding acquisition, Conceptualization. **Chang Cai:** Methodology, Validation, Software, Data curation. **Jiqi Li:** Methodology, Investigation, Data curation. **Yiting Chen:** Methodology, Investigation. **Jiaheng Chen:** Validation, Visualization. **Qian Zhang:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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Conflict of interest

The authors declare no competing interests.

Availability of data and materials

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

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Figures

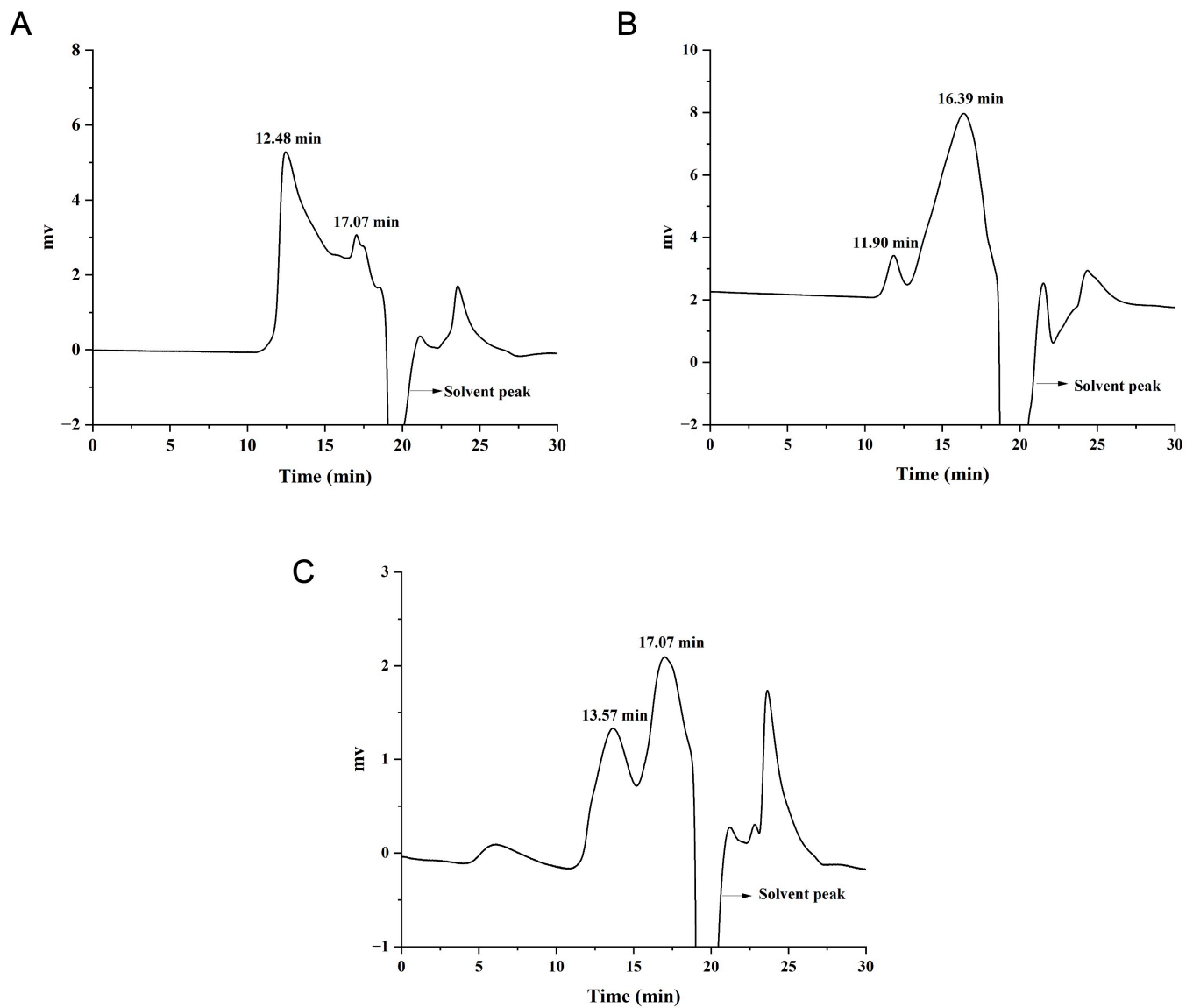


Figure 1

The HPGPC spectra of *D. delavayi* fruit polysaccharide fractions. (A) The HPGPC spectrum of DDP-40, (B) The HPGPC spectrum of DDP-60, (C) The HPGPC spectrum of DDP-80.

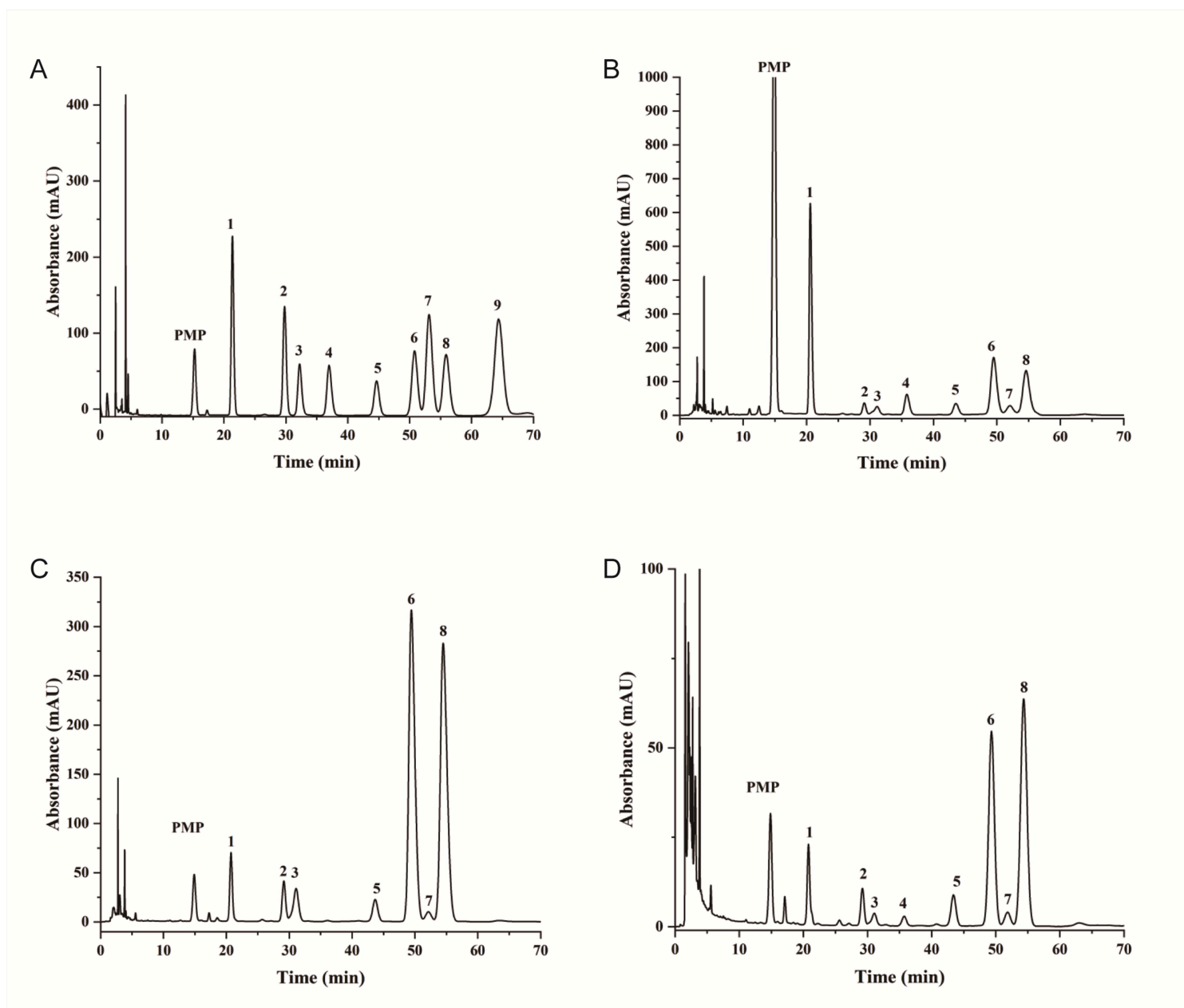


Figure 2

The monosaccharide components of *D. delavayi* fruit polysaccharides. (A) The HPLC of the monosaccharide mixture, (B-D) The HPLC chromatograms of DDP-40, DDP-60 and DDP-80 monosaccharide component are presented in sequence.

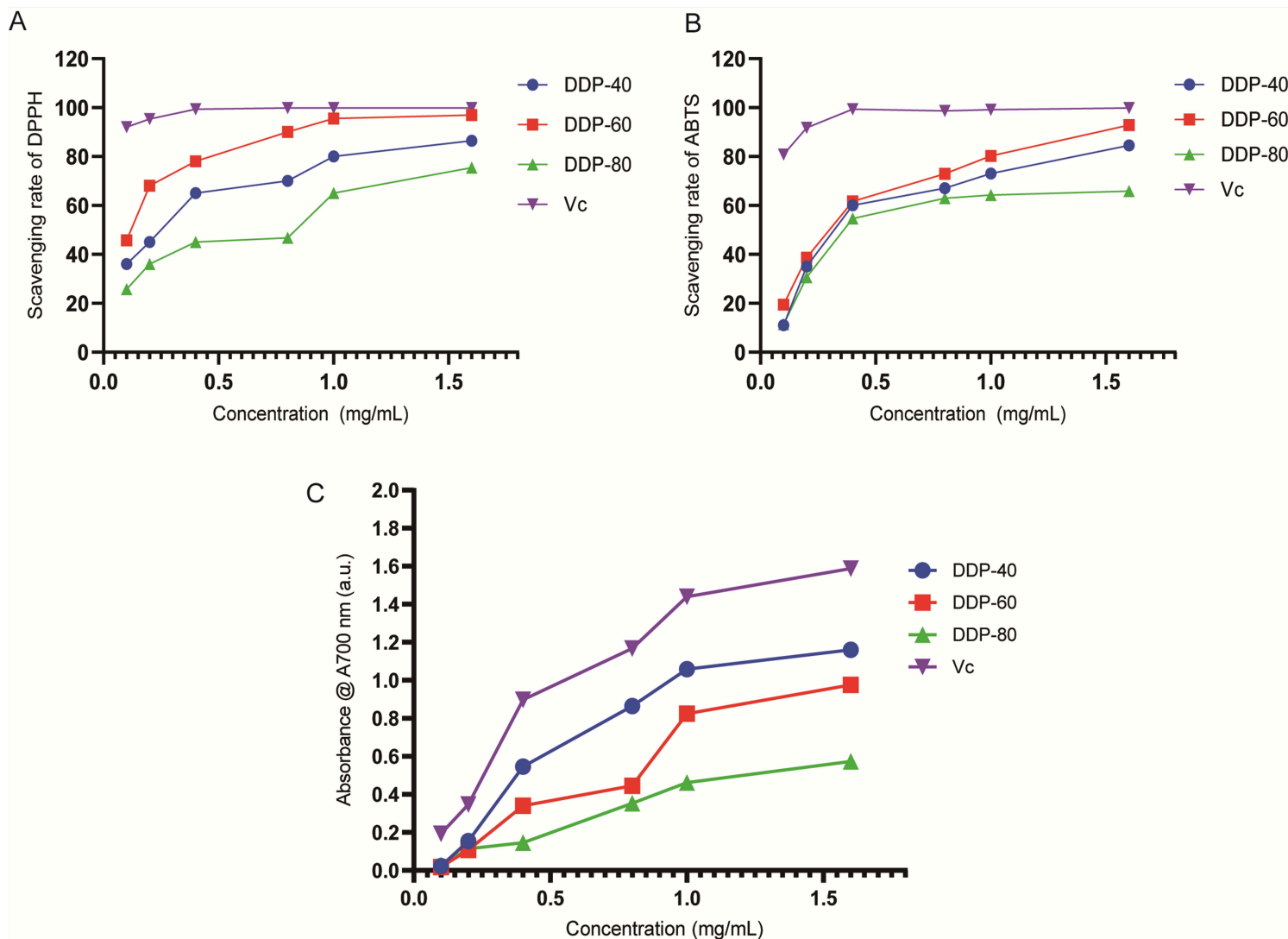


Figure 3

The antioxidant capacity of *D. delavayi* fruit polysaccharide fractions. (A) The free radical scavenging ability of DPPH, (B) The free radical scavenging ability of ABTS, (C) The total reducing capacity of Fe^{2+} .

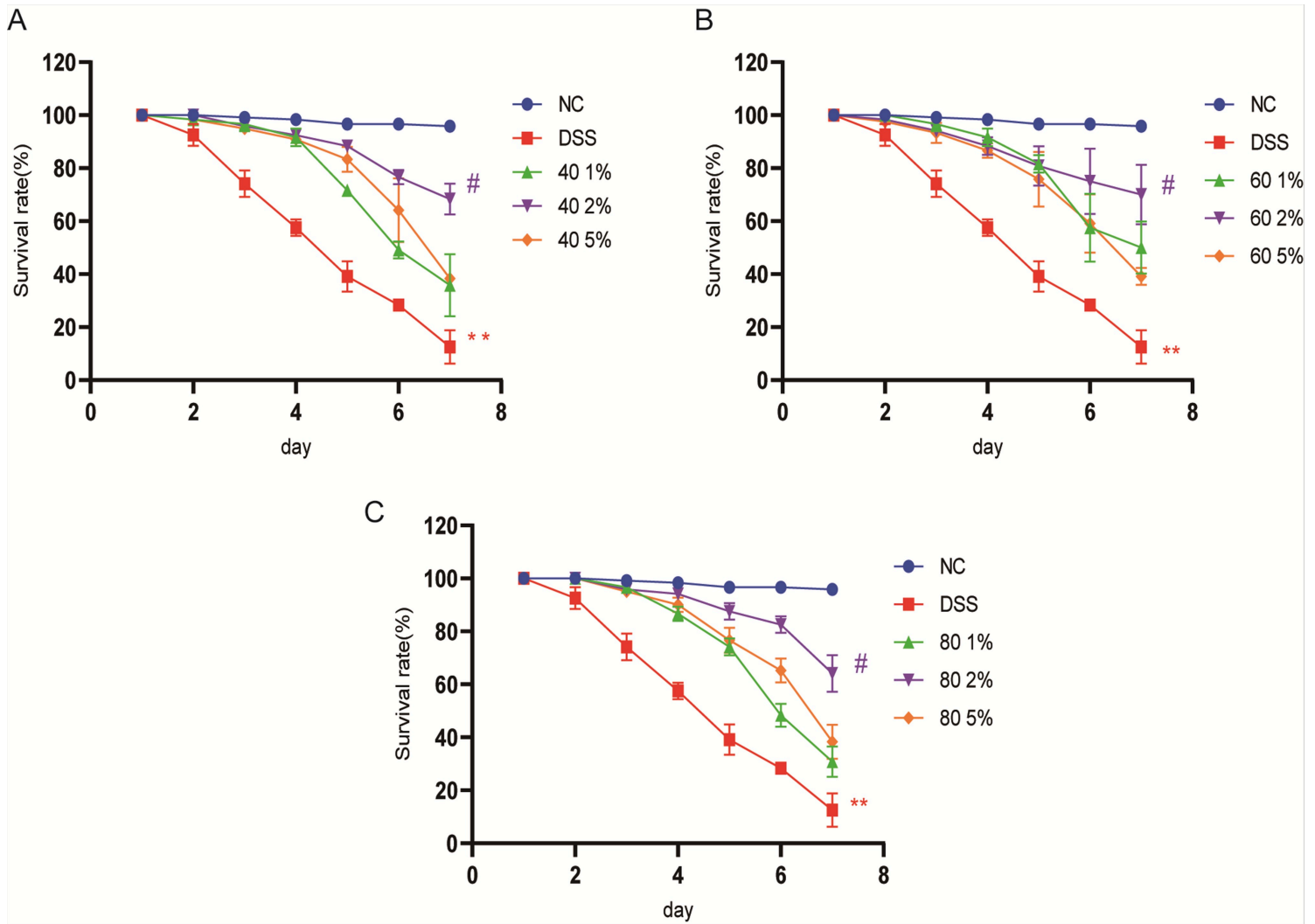


Figure 4

The impact of *D. delavayi* fruit polysaccharide fractions on the survival rate of fruit flies. (A-C) The survival rates of the fruit flies were affected differently by various concentrations of DDP-40, DDP-60, DDP-80, Separately. # indicates that there is a significant difference between 2% DDP-40, DDP-60, DDP-80 and DSS, #P ≤ 0.05, ** indicates that there is a significant difference between DSS and NC group, with **P ≤ 0.01.

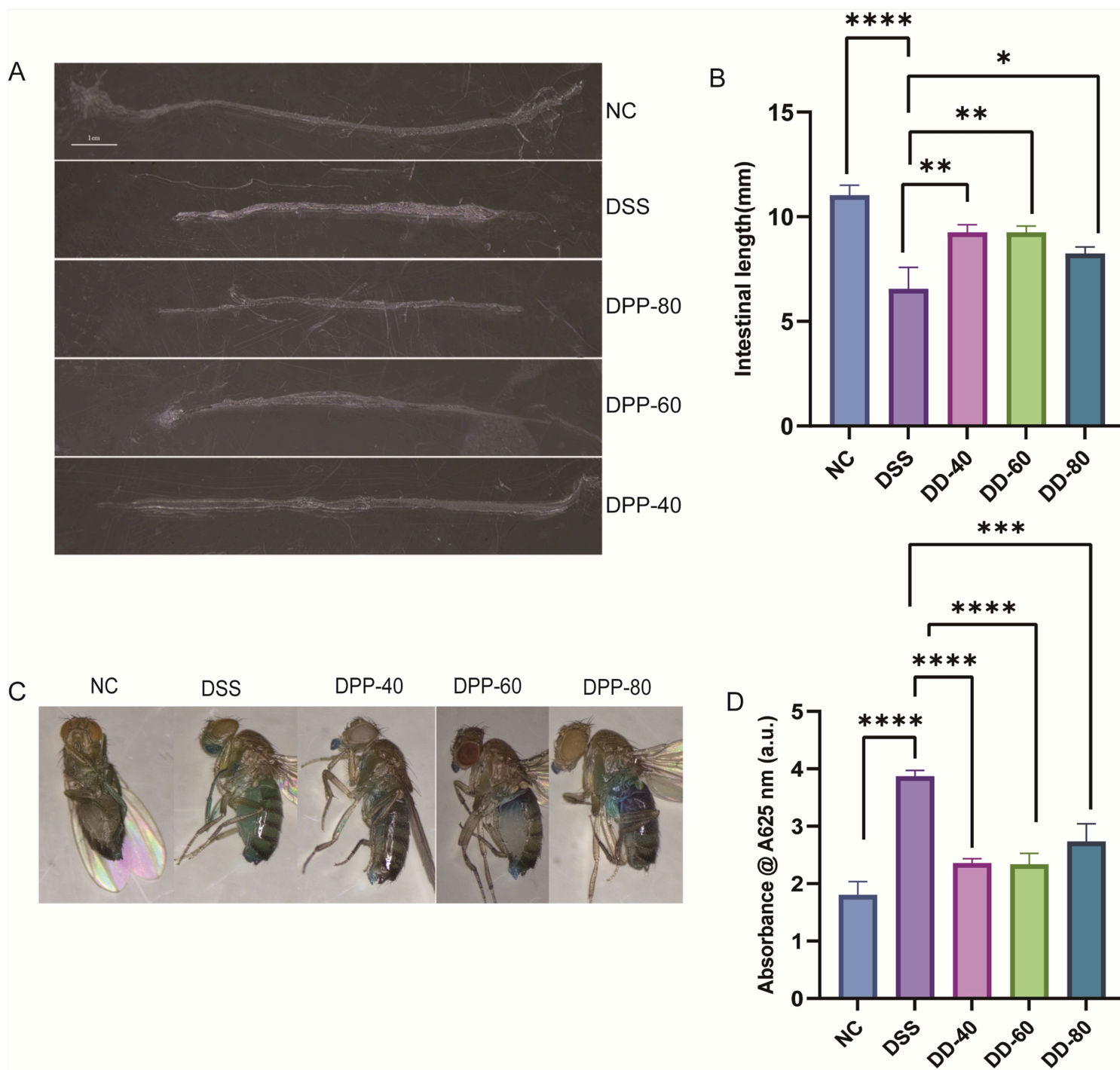


Figure 5

The influence on the morphology and permeability of the fruit fly's intestinal tract. (A) The intestinal morphology of fruit flies in different groups, (B) Analysis of the length of the fruit fly's intestine ($n = 8$), (C) The overall blue staining situation of the fruit, (D) The absorbance values at 625 nm after homogenization of each group of fruit flies ($n = 8$). $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ VS. the DSS group.

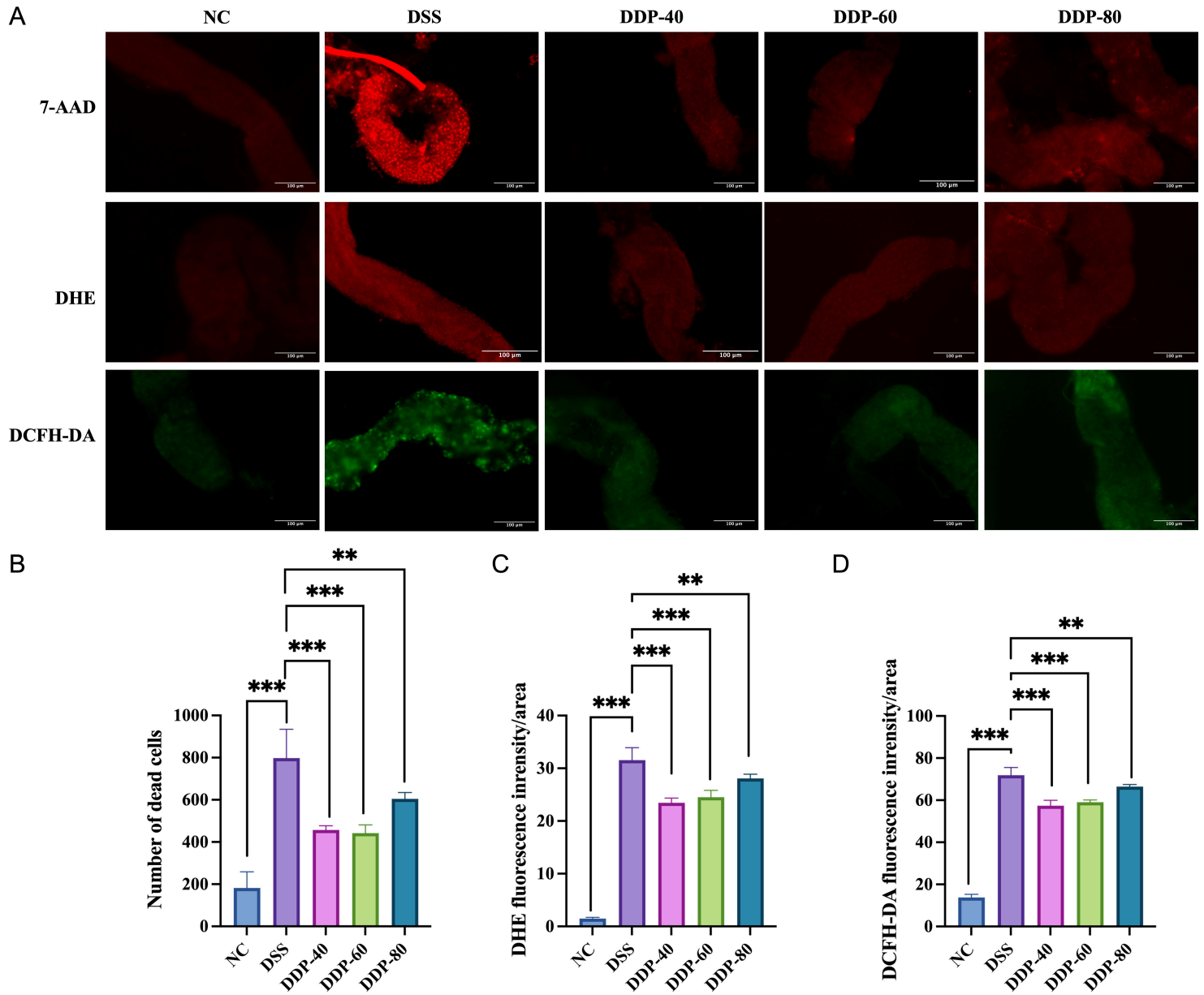


Figure 6

The impact on the apoptosis and ROS levels of intestinal epithelial cells.

(A) 7-AAD, DCFH-DA and DHE staining of *Drosophila* intestines, (B) Number of dead cells (n = 8), (C and D) Fluorescence intensity of ROS levels (n = 8). ** $p < 0.01$, *** $p < 0.001$ VS. the DSS group.

Supplementary Files

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