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Original article

Total ginsenosides promote the IEC-6 cell proliferation via affecting the regulatory mechanism mediated by polyamines

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ABSTRACT

Epithelial cell proliferation has been demonstrated to be a critical modality for mucosal repair after gastrointestinal mucosal injury. This research aimed to investigate the effect of total ginsenosides upon the proliferation of intestinal epithelial cells (IEC-6), and elucidate its potential mechanisms through polyamine-regulated pathway including the expression of proliferation-related proteins. Total ginsenosides (PGE3) were extracted from *Panax ginseng*, a Chinese herbal medicine, whose chromatogram was obtained by high performance liquid chromatographic method with evaporative light scattering detection (HPLC-ELSD). The cell proliferation, cell cycle distribution and the level of c-Myc, RhoA, Cdk2 proteins were detected to determine the effects of PGE3 at 25, 50 and100 mg/l doses on IEC-6. Furthermore, rats model of intestinal mucosal injury were induced by the subcutaneous injection of indomethacin, and the effect of *Panax ginseng* aqueous extracts (PGE1) on intestinal mucosal injury was observed. PGE3 could promote IEC-6 cell proliferation, reduce the proportion of G0/G1 phase cells and elevate the proportion of G2/M + S phase cells, and revert the proliferation and cell cycle arrest induced by DFMO (DL-a-difluoromethylornithine, an inhibitor of polyamines synthesis). PGE3 exposure enhanced the level of c-Myc, RhoA and Cdk2 proteins, and reversed the inhibition of these proteins expression induced by DFMO. The results of gross and pathological scores showed administration of PGE1 significantly alleviated intestinal mucosal injury of rats. Our findings indicate that total ginsenosides promoted the IEC-6 proliferation presumably via its regulation on cell cycle and the expression of proliferation-related proteins regulated by polyamines, and provided a novel perspective for exploring the repair effect of *Panax ginseng* upon gastrointestinal mucosal injury.

1. Introduction

Gastrointestinal mucosal injury is a common pathological manifestation in spleen-deficiency syndrome [a classical syndrome in traditional Chinese medicine (TCM) theory] and many gastrointestinal diseases such as inflammatory bowel disease (Zhang et al., 2020). Mucosal repair after gastrointestinal injury is an important way to restore and maintain gastrointestinal barrier function (Farré et al.,2020), of which the regulatory defects may be the pathogenesis basis of stress ulcer, peptic ulcer, inflammatory bowel disease as well as the anorexia, abdominal distension (TCM symptoms) and so on (McElrath et al., 2021; Flemming et al., 2020; Birkl et al., 2019). Gastrointestinal mucosal integrity is the morphological basis of the mucosal barrier(Chelakkot et al.,2018), and the ability to repair the gastrointestinal mucosa after injury is an important factor in maintaining its integrity, therefore, the role of repairing gastrointestinal mucosa after injury

Abbreviations: IEC-6, intestinal epithelial cells; DFMO, DL-α-difluoromethylor nithine; Cdk2, cyclin-dependent protein kinase 2; RhoA, Ras homolog family member A; PGE, *Panax ginseng* extracts; PGE1, *Panax ginseng* aqueous extracts; PGE3, total ginsenosides; RQSS, reinforcing Qi strengthening spleen.

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has an important pathophysiological significance (Odenwald and Turner, 2013). The repair process is regulated by a variety of cellular factors including polyamines (Rao et al., 2020). Polyamines (spermine, spermidine and putrescine) are the central focus of many complex signal pathways that regulate the different functions of epithelial cells (Grosheva et al., 2020). Besides, polyamines play a potent role in the repair process after the gastrointestinal mucosal injury, such as facilitating cell proliferation, migration, differentiation and regulating cell junctions (Gao et al., 2013).

An increasing body of evidences have confirmed that the Chinese medicine of reinforcing Qi strengthening spleen (RQSS) could protect the gastrointestinal barrier by promoting the repair of injured gastrointestinal mucosa. Our earlier animal experiments had proved that the mechanism of RQSS Chinese medicine on the repair of gastrointestinal mucosal injury was related to the increasing level of polyamines. For example, the extracts from *Astragalus membranaceus*, *Atractylodes macrocephala*, *Codonopsis pilosula or Glycyrrhiza uralensis* (TCM of RQSS) could prevent gastric mucosal injury caused by stress ulcer in rats, which was presumably associated with the elevated content of polyamines in the gastric mucosa (Nian, 2013). The extracts of Sijunzi decoction (*Panax ginseng* is the main medicine of this formula) had preventive and therapeutic effects on indomethacin-induced gastrointestinal mucosal injury probably by stabilizing the mucosal barrier via upregulating polyamines content and expression level of tight junctions, adherens junctions proteins (Wang et al., 2018a; Wang et al., 2018b). We found previously in vitro that the extracts from *Astragalus membranaceus*, *Atractylodes macrocephala*, *Codonopsis pilosula, Glycyrrhiza uralensis* or Sijunzi decoction promoted IEC-6 migration and cell proliferation, differentiation and intercellular junctions by regulating polyamines, which suggested that the effect of Chinese medicines of RQSS on the repair of gastrointestinal mucosa after injury is tied up with the polyamines (Li et al., 2010a; Zeng et al., 2018; Song et al., 2014; Li et al., 2015a; Li et al., 2015b; Li et al., 2016; Zeng et al., 2017; Tu et al., 2016a; Tu et al., 2016b; Shi et al., 2019; Li et al., 2010b; Wu et al., 2017).

Panax ginseng (*Panax ginseng C. A. Mey*), the representative of herbal medicines of RQSS, has been widely employed in Eastern Asia for thousands of years. It is used to alleviate digestive conditions via invigorating Qi, promoting fluid production and nourishing blood, and is the main medicine of many tonifying formulas such as Dushen decoction, Sijunzi decoction, Buzhong Yiqi decoction and so on. The active components of *Panax ginseng* include saponins, polysaccharides, volatile oils, flavonoids, peptides, trace elements and so on (Mancuso and Santangelo, 2017), of which ginsenoside is taken into consideration as one of the major active components (Kim et al., 2017). Ginsenoside effectively ameliorated gastrointestinal digestion and absorption function of animals with spleen deficiency (a syndrome of TCM), had antioxidant and anti-inflammatory properties (An et al., 2021) and promoted the renewal of IECs to maintain the integrity of gastrointestinal mucosa by accelerating gastrointestinal mucosal mucus secretion and blood flow, augmenting the number of microvilli, mitochondria, endoplasmic reticulum, and increasing mitotic index of intestinal epithelial cells (Peng and Lei, 1997; Peng and Lei,1999). However, there are few reports about the underlying mechanisms of ginsenoside in protecting gastrointestinal mucosa. The purpose of this research was to observe the effect of total ginsenosides upon the proliferation of IEC-6 cells respectively in culture media containing DFMO or not, and to reveal its potential mechanisms of action by analyzing changes of cell cycle and the expression of proliferation-related proteins (c-Myc, Cdk2, RhoA) regulated by polyamines. Meanwhile, the repair effects of ginseng aqueous extracts on intestinal mucosal injury induced by indomethacin in rats and its pharmacodynamic material basis were studied in vitro.

2. Materials and methods

2.1. Materials

Panax ginseng (Renshen), was provided by Guangzhou Tong Kang Pharmaceutical Co. Ltd and was identified by associate professor Tong JY from the Department of Chinese medicine identification in Guangzhou University of Chinese Medicine. IEC-6 cells were purchased from the ATCC (No. 863139935, VA, U.S.A). In addition, indomethacin, putrescine and DFMO were obtained from Sigma-Aldrich (MO, U.S.A). Fetal bovine serum (FBS), DMEM and streptomycin, penicillin were provided by Gibco (Mass, U.S.A). Anti-c-Myc, Cdk2, RhoA, GAPDH antibodies and secondary antibody were purchased from Abcam (Cambridge, U.K).

2.2. Preparation of PGE1 (Panax ginseng aqueous extracts)

Briefly, a total of 600 g of *Panax ginseng* was chopped and then immersed in twelve times the volume of pure water for 2 h. Subsequently, the mixture was decocted for 2 h, recovered filtrate. These operations were repeated two times. The first and second decoction were pooled together, concentrated, and freeze-dried to produce PGE1.

2.3. Preparation of PGE2 and PGE3 (total ginsenosides extracts of Panax ginseng)

A total of 300 g of dried *Panax ginseng* powder was added with10-times volume of 80% ethanol, and then the mixture was refluxed for 1.5 h at 60 °C, which was repeated in three times. The mixtures of ginseng and 80% ethanol were filtered and the filtrate was recovered. All decoction were pooled together and concentrated to a final volume of 450 ml. The decoction was extracted with petroleum ether for 3 times and water-saturated n-butanol for 6 times to produce PGE2. The extracts were dissolved in a small amount of methanol by ultrasonic after it was concentrated and freeze-dried. Then, 80% ethanol eluent was collected when the extracts passed through D101 macroporous resin, concentrated and freeze-dried to obtain PGE3 (total ginsenosides samples).

2.4. Properties of PGE (extracts of Panax ginseng)

2.4.1. Determination of ginsenoside

Vanillin-sulfuric acid assay, which was described by Hiai S (Hiai et al., 1975), was conducted to determine the content of ginsenoside in PGE2 and PGE3 with ginsenoside Re as a standard.

2.4.2. Chromatographic detection of PGE3

HPLC system (Grace, USA) equipped with ELSD 3300 (Alltech Associates, USA) was employed to determine the purity of total ginsenosides. Briefly, a total of 20 ul PGE3 was dissolved in methanol (2 mg/ml) and applied to the column after being filtered by 0.22 μm filter. The temperature of the column was maintained at 30 °C, and the mobile phase was composed of A (acetonitrile) and B (water), gradient elution was performed on a Ecosil C18 column (5 μm, 4.6 mm × 250 mm) at the flow rate of 1 ml/min. Finally, the data that consisted of compound Molecular Weight (comp MW), Retention Time (RT), peak intensity and observations (samples) were edited by excel 2019 software.

2.5. In vitro investigation

2.5.1. Cell culture

IEC-6 cells were routinely cultured in DMEM medium with 1% antibiotics (streptomycin,penicillin) and 5% FBS at 37 °C under a humidified atmosphere of 95% air and 5% CO_2 as described previously (Shi et al., 2019).

2.5.2. MTT assay

Cell proliferation were detected by MTT assay according to the previous research (Shi et al., 2018). IEC-6 cells (5×10^4 cells/ml) were seeded in 96-well plates for 24 h in the above conditions and incubated without FBS for 24 h, and subsequently cultured with DMEM medium containing 0.5% FBS. Furthermore, IEC-6 cells were treated with control, PGE3 (25, 50 and100mg/l) and putrescine (20 μmol/l, PUT) respectively, supplemented with DL-α-difluoromethylornithine (2.5 mmol/l) or not at appropriate time. After 48 h of stimulation, MTT solution with a concentration of 5 mg/ml (20 ul, Sigma) was added into each well for another 4 h and then replaced with DMSO (150 μl, Sigma, U.S.A). At last, the absorbance values at 490 nm were measured by a microplate reader.

2.5.3. Flow cytometry assay

The distribution of cell cycle was determined using flow cytometry assay in CytomicsTM FC 500 flow cytometry system (Beckman Coulter, Inc.,USA). IEC-6 cells were harvested and fixed with precold anhydrous ethanol for 24 h at 4 °C. After being washed with PBS and centrifuged, cells were treated with 100 μl RNaseA (Biofroxx, Germany) at 37 °C for 30 min, subsequently stained with PI (400 ul, Biofroxx) solution in the dark for 30 min. Finally, the distribution of cell cycle was analyzed with MultiCycle software.

2.5.4. Western blotting analysis

IEC-6 cells were lysed with RIPA buffer containing PMSF (Beyotime, China), and subsequently the concentration of proteins were quantified with BCA assay. Moreover, equal amount of proteins were separated with SDS-PAGE gel, and subsequently transferred onto the PVDF membrane. The membrane was blocked in TBST containing 5% non-fat milk for 1 h, followed by incubating with primary antibodies to c-Myc(1:10000), RhoA(1:5000), Cdk2 (1:5000), GAPDH(1:10000) at 4 °C overnight. Subsequently, the membrane was incubated with goat polyclonal antibody to rabbit IgG as the secondary antibody (1:5000) for 1 h. GAPDH was employed as an internal control. Blots were quantified through Image Lab software.

2.6. In vivo investigation

2.6.1. Animal experiments

Adult male Sprague-Dawley rats (180 ~ 220 g, SPF) were purchased from the Laboratory Animal Center in Guangzhou University of Chinese Medicine (No.44005800008169). Rats were reared five individuals each cage with water and food freely available in the SPF animal room provided by Guangzhou University of Chinese Medicine under a 12 h dark-light cycle (No.00200181). All animal procedures were performed strictly in accordance with the "Guiding Principles for Research Involving Animals and Human Beings" as well as were approved by the committee of animal ethics in Guangzhou University of Chinese Medicine.

After 5-day acclimatization period, animals were randomly assigned to four groups: normal group (n = 6), model group (n = 16), PGE1 5 g/kg group (n = 12) and PGE1 15 g/kg group (n = 12). Intestinal mucosal injury was induced by indomethacin (6 mg/kg, i.h., Sigma) once daily for 4 days in the model group and PGE1 groups, while rats of the normal group were given the same volume of sterile saline. In fact, rats in each group were treated the corresponding drug or sterile saline by intragastric gavage 1 h before the models were induced. Rats in PGE1 groups were treated with PGE1 (5 g/kg or 15 g/kg) once daily for seven days, while rats in other groups were supplemented with the equal volume of sterile saline.

2.6.2. Gross morphology of small intestine

Rats were sacrificed twenty-four hours after the last administration, the mesentery was separated from the intestine after the abdomen was opened, and then the whole small intestinal tissue was taken and cut longitudinally. The gross morphology of whole small intestine was observed and scored according to the severity of adhesion and ulcer respectively (Zhu et al., 1998), as follows:(1) adhesion grading scale:0 = normal, no adhesion;1 = mild or moderate adhesion, the small intestine could be separated from other tissues with a little force;2 = severe adhesion. (2) ulcer grading scale: 0 = normal, no ulcer; 1 = hyperemia and edema, but no ulcer on local mucosal surface; 2 = mild ulcer, with no significant mucosal edema or hyperemia or other inflammatory manifestations; 3 = single ulcer with inflammation; 4 = ulcers at two or more sites, with inflammation, and the diameter of the ulcer < 1 cm; 5 = ulcers at two or multiple sites, with inflammation, and the diameter of at least one ulcer > 1 cm; others: the diameter of ulcer and/or inflammation > 2 cm, and the score was increased by 1 point for each increase of 1 cm in the lesion area. The total score was the sum of the two.

2.6.3. Histopathological evaluation

Small intestine tissues were collected, washed with pre-cooled PBS, fixed in 4% neutral paraformaldehyde, then dehydrated, embedded in paraffin, and finally cut into 3um sections that could be stained with hematoxylin and eosin (H&E). The pathological changes of intestinal mucosal injury was observed under microscope and divided into 6 grades according to Chiu et al. (Chiu et al., 1970): 0 = normal intestinal mucosal villi; 1 = development of subepithelial Gruenhagen's space at the apex of villi, usually with capillary hyperemia; 2 = extension of the subepithelial space, often with moderate edema of lamina propria and dilatation of central lacteal; 3 = obvious edema of lamina propria, degeneration and necrosis of intestinal epithelial cells, with a few denuded villi tips; 4 = denuded villi with congested capillaries and exposed the lamina propria; 5 = loss of villi, disintegration of lamina propria, development of hemorrhage or ulceration. The pathological score of each field of per specimen was the mean of 10 consecutive objects in 3 sections, which was carried out by researchers who were blinded to the study. The more serious the mucosal injury, the higher the score.

2.7. Statistical analysis

All data that had obtained were presented as the means ± SD. Mean values between different groups were compared by applying one-way analysis of variance (ANOVA) or Kruskal-Wallis H test, followed by SNK test or Mann-Whitney *U* test. The P value < 0.05 was taken into consideration as statistical significance. Statistical analysis of all data was performed by applying SPSS version 21.0.

3. Results

3.1. Yield and property of PGE

The yields of PGE1, PGE2 and PGE3 extracts were 43.8%, 3.89% and 2.12% respectively. The content of saponins in PGE2 and PGE3 was 50.8% and 92.2% respectively. And HPLC-ELSD of PGE3 was shown in Fig. 1.There were three main absorption peaks in

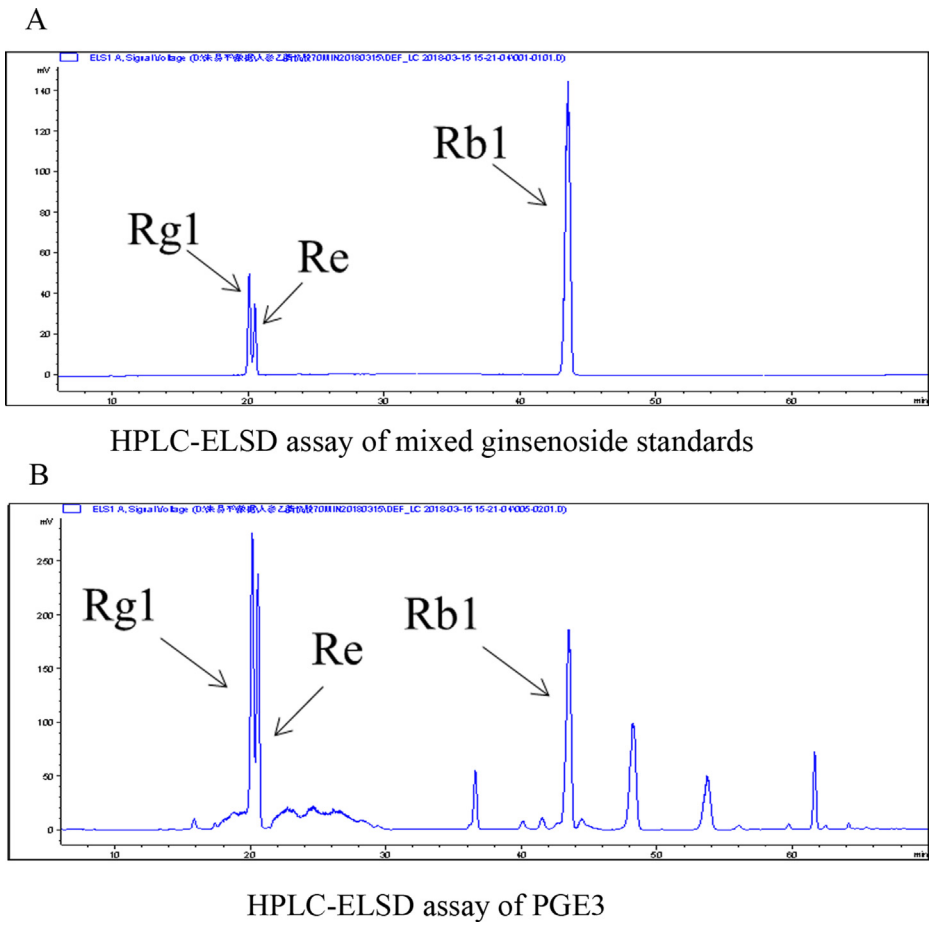

Fig. 1. HPLC-ELSD of PGE3. (A) HPLC-ELSD assay of mixed ginsenoside standards; (B) HPLC-ELSD assay of PGE3.

PGE3 with the same retention time as ginsenoside standard samples, indicating that PGE3 samples mainly contained ginsenoside Re, ginsenoside Rg1 and ginsenoside Rb1.

3.2. In vitro evaluation

3.2.1. Effect of PGE3 on cell proliferation in vitro

In the research, we used the MTT method to detect effect of PGE3 on IEC-6 cell proliferation. The results revealed that the promoting effect of PGE3 (50 mg/l, 100 mg/l) on IEC-6 cell proliferation was more significant than that of control group ($P < 0.05$, Fig. 2A). In addition, compared with the control group, polyamines depletion by DFMO led to significant reduction in the number of cells ($P < 0.01$). The treatment with PGE3 (50 mg/l, 100 mg/l) could reverse the arrest of IEC-6 cell proliferation induced by DFMO, which indicated that the effect of PGE3 on IEC-6 cell proliferation was tied up with polyamines ($P < 0.05$, Fig. 2B).

3.2.2. Effect of PGE3 upon cell cycle progression in vitro

Compared to control group, the treatment with PGE3 (50 mg/l, 100 mg/l) stimulated the cell cycle progression by promoting transition from G0/G1 to G2/M + S phase ($P < 0.05$, Fig. 3A,B,C). Depletion of intracellular polyamines by DFMO significantly enhanced the ratio of G0/G1 phase cells and reduced ratio of G2/M + S phase cells, compared with the control group ($P < 0.05$). What's more, cell cycle arrested by DFMO could be almost reversed by concomitant treatment with PGE3(50 mg/l, 100 mg/l), suggesting that the effect of PGE3 upon cell cycle progression was tied up with polyamines (Fig. 3D,E,F).

3.2.3. Effect of PGE3 on expression of cell proliferation-related proteins

To explore the molecular mechanisms of PGE3 on IEC-6 cell proliferation, the expression of c-Myc, RhoA and Cdk2 proteins were detected. As exhibited in the Fig. 4, the expression level of c-Myc, RhoA and Cdk2 proteins in PGE3 (50 mg/l, 100 mg/l) groups were much higher than that in control group ($P < 0.05$), suggesting that PGE3 might promote cell proliferation by elevating the expression level of c-Myc, RhoA and Cdk2 proteins. Compared with control group, polyamines depletion by DFMO downregulated the level of c-Myc, RhoA and Cdk2 proteins ($P < 0.05$), which were attenuated by PGE3 (50 mg/l, 100 mg/l. Fig. 5).

3.3. In vivo evaluation

3.3.1. Effect of PGE1 upon small intestinal mucosal injury induced by indomethacin in rats

As demonstrated in the Fig. 6, rats in the model group represented severe small intestinal mucosal injury characterized by severe intestinal adhesions, intestinal mucosal congestion and edema, and multiple ulcers, while there was no obvious lesion in the normal group (Fig. 6A). PGE1 significantly reduced small intestinal gross morphology score in rats as compared with the model group ($P < 0.05$, Fig. 6A,B). The small intestinal mucosa was intact and the intestinal villous epithelium was not exfoliated in normal group, while rats in model group presented with obvious damage of small intestinal mucosa such as degeneration and necrosis of intestinal epithelium, exfoliation of villi, disintegration of lamina propria and multiple ulcers (Fig. 6D). Compared with the model group,

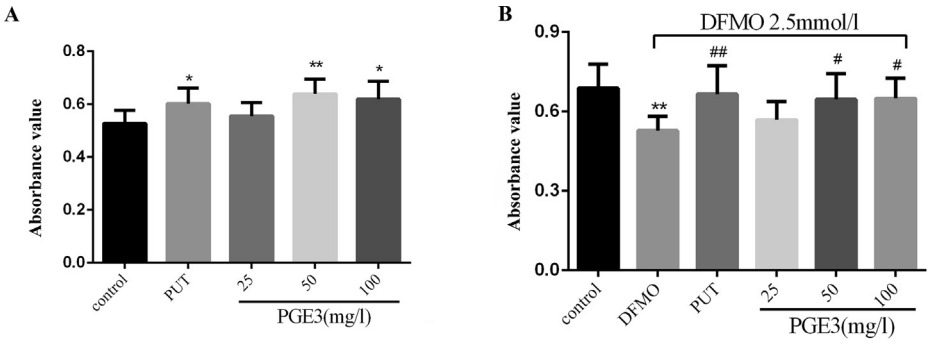

Fig. 2. Effect of PGE3 upon cell proliferation in vitro. (A) The absorbance values of IEC-6 cell under different treatments; (B) The absorbance values of IEC-6 cell under different treatments containing DFMO. (*P < 0.05 and **P < 0.01, compared with the control group; #P < 0.05 and ##P < 0.01, compared with the DFMO group. n = 3) Data were analyzed by one-way ANOVA followed by SNK-test.

PGE1 15 g/kg group showed better histopathological appearance and lower pathology score (Fig. 6C,D).

4. Discussion

The gastrointestinal mucosa, the most rapidly self-renewing tissue in the human body, completely renewed every 3–4 days under physiological condition through the dynamic balance of IECs proliferation and apoptosis. Cell proliferation is involved in the fundamental process of mucosal restitution after wounding, whereas epithelial cell proliferation is known to be dependent on the cell cycle. The cell cycle is controlled by many regulator molecules, of which polyamine-regulated c-Myc (transcription factor), Cdk2 (cyclin-dependent kinase 2) and RhoA (RhoGTP enzyme) are positive regulators (Weiger and Hermann, 2014; Liu et al., 2009).

Intestinal epithelial cells could quickly repair injured mucosa through modifying the patterns of expressed genes. The activation of transcription factors is essential to control a range of specific genes expression that regulate cell proliferation, migration, junctions and epithelial barrier. c-Myc as a transcription factor is an important regulator of cell proliferation. Repair of gastric and duodenal mucosal injury after stress is bound up with the enhanced level of the c-Myc gene following elevated content of intracellular polyamines, whereas the restrain of c-Myc expression caused by intracellular polyamines depletion leads to the decrease of cell proliferation and the delay of mucosal healing (Liu et al., 2012). Epithelial cell proliferation stimulated by c-Myc exerts a significant role in promoting intestinal mucosal repair via polyamines-regulated pathway. The depletion of polyamines induced by DFMO could decrease c-Myc gene transcription and cell proliferation, which could be reversed by exogenous polyamines, suggesting that the c-Myc induced by polyamines exerts a significant influence on stimulation of IECs proliferation (Liu et al., 2005). c-Myc also implicates in the modulation of cell cycle. It could trigger the entry in cell cycle progression of quiescent cells by regulating target genes encoded (Chan et al., 2010) and antagonizing the activity of cell cycle inhibitors (Bretones et al., 2015; Butt et al., 2005; Ohtani et al., 1995), inducing DNA replication and involving in the mitotic control (Zhang et al., 2019; Kim et al., 2018).

Cdk2, a member of cyclin kinase family, is not only one of the core factors in regulating cell cycle but also involves in stimulating cell proliferation via facilitating the accurate transition through G1 phase to S phase and from S phase to G2/M phase of the cell cycle. The depletion of intracellular polyamines by exposure to DFMO could inhibit cell proliferation, and the percentages of IEC-6 cells distributed in the G1 phase were enhanced while the percentages in the G2/M and S phase were reduced, indicating that most of the intestinal epithelial cells cycle were arrested in the G1 phase. Meanwhile, Cdk2 expression was also inhibited (Ray et al., 2001). RhoA, Rho GTP enzyme, not only participates in driving cell migration, but also exerts a significant influence on the regulation of cell proliferation. Overexpression of RhoA significantly stimulated cell proliferation, increased the expression of Cdk2 protein and promoted G1/S transition in IEC-6 cells, while these positive effects of activating RhoA on cell proliferation could be significantly inhibited by polyamines depletion (Guo et al., 2003).

Ginseng is mainly used in the form of aqueous decoction in traditional clinical applications. In this study, aqueous extract of ginseng was selected as the test drug for animal experiments to observe the preventive and curative effects of ginseng herbs on indomethacin-induced small intestinal mucosal injury, and the main points for consideration include: the aqueous extract of ginseng is consistent with the traditional use of ginseng in dosage form, and we observed the protective effect of ginseng herbs on intestinal mucosa at the animal level; in addition, the aqueous extract of ginseng also conforms to the characteristics that the action of the original herb is directly observed in animal experiments, which provides a basis for further extraction of different components of ginseng and exploration of its pharmacodynamic substances. Total ginsenosides was selected as the test drug for cellular experiments to observe the effects of total ginsenosides on the proliferation and cell cycle of intestinal epithelial cells, and the main points for consideration include: the composition of total ginsenosides is relatively clear, we could explore the possible potential mechanisms of ginseng fractions promoting mucosal repair after injury at the cellular level, and further explored the specific pharmacodynamic substance of ginseng on the basis of animal experiments. Ginsenosides and ginseng polysaccharides are the main active components of ginseng. It is reported that ginsenosides could increase the number of microvilli, mitochondria, and endoplasmic reticulum in small intestinal epithelial cells of rats with spleen deficiency model, which indicated that ginsenosides have a protective effect on gastrointestinal mucosa (Peng and Lei, 1997). In another animal experiment, we chose ginseng polysaccharides as the test drug to observe its effect on indomethacin-induced mucosal injury in the small intestine of rats. The results showed that ginseng polysaccharides could reduce the degree of ulcer damage, increase the Ca^{2+} content of small intestinal mucosa (study results to be published). And the results of Brdu expression showed that ginseng polysaccharides could promote the migration of small intestinal epithelial cells in rats (study results to be published). These results suggested that the efficacy results of the active fraction of ginseng (ginseng polysaccharide) on the indomethacin-induced small intestinal mucosal injury model in rats were similar to those of the ginseng herb.

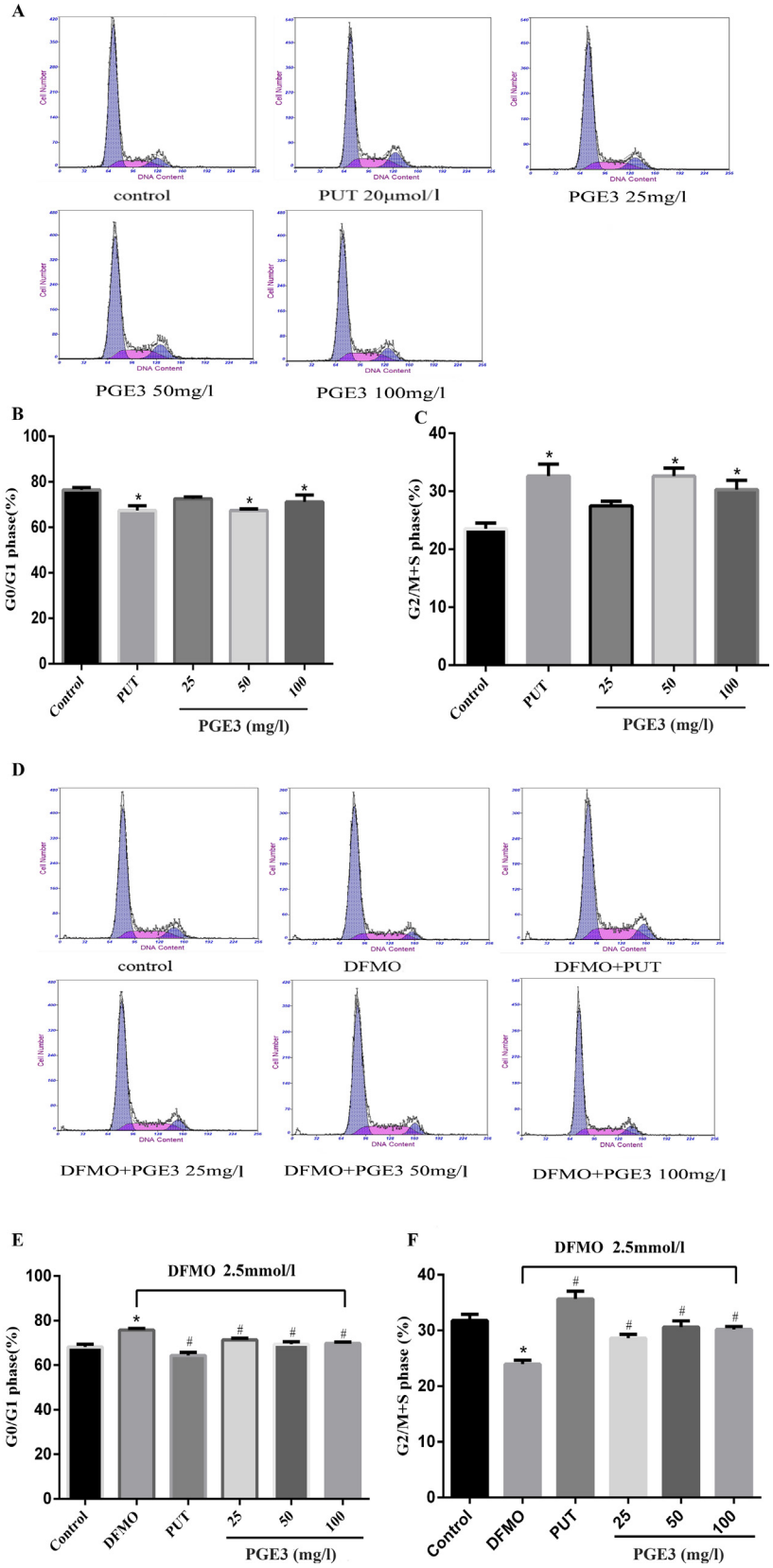

Fig. 3. Effect of PGE3 upon cell cycle in vitro. (A)The cell cycle distribution with different treatments analyzed by flow cytometry assay; (B) The percentage of G0/G1 phase IECs in different groups; (C) the percentage of G2/M + S phase IECs in different groups; (D) The cell cycle distribution analyzed by flow cytometry assay with different treatments containing DFMO; (E) The percentage of G0/G1 phase IECs in different groups containing DFMO; (F) the percentage of G2/M + S phase IECs in different groups containing DFMO. (*P < 0.05, compared with the control group; #P < 0.05, compared with the DFMO group. n = 3) Data were analyzed by one-way ANOVA followed by SNK-test.

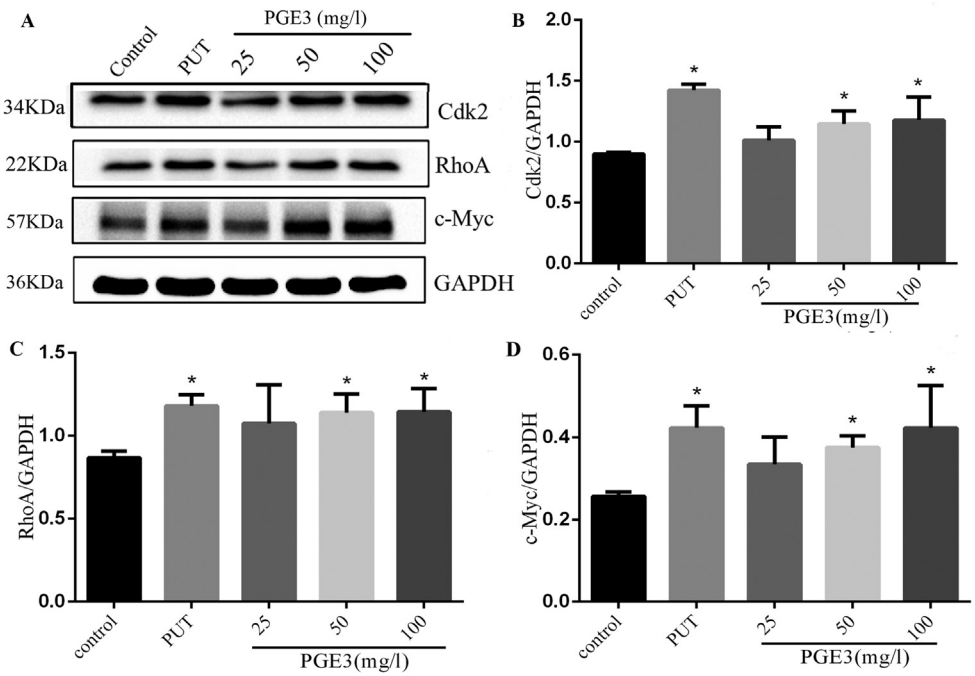

Fig. 4. Cell proliferation-related proteins levels assessment in normal culture. (A)Western blotting analysis of Cdk2, RhoA and c-Myc expression in different treatments; The bar graph reflected Cdk2(B), RhoA(C) and c-Myc(D) proteins expression in each group. (*P < 0.05, compared with the control group. n = 3) Data were analyzed by one-way ANOVA followed by SNK-test.

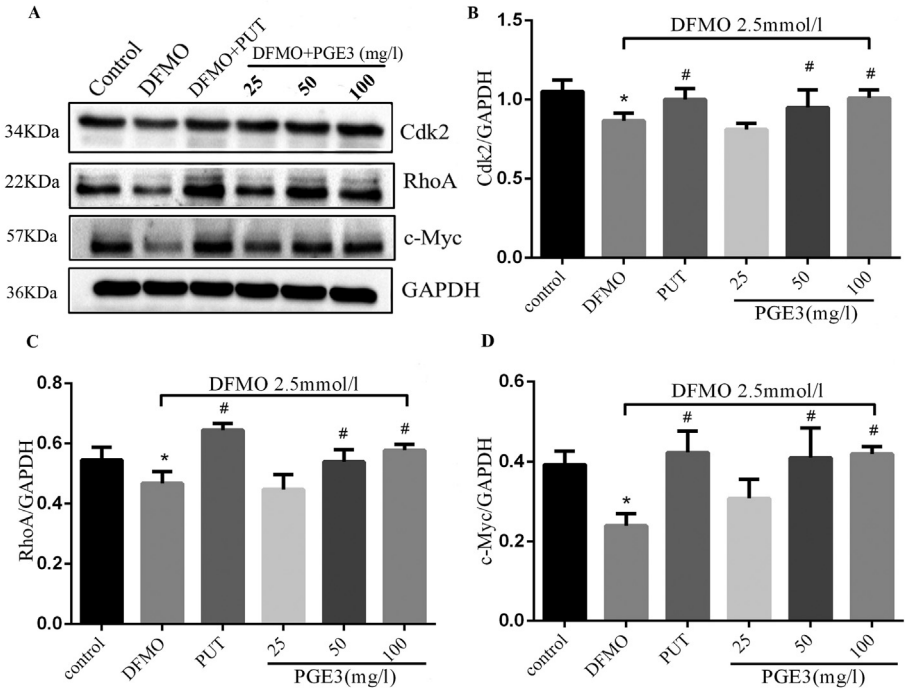

Fig. 5. Cell proliferation-related proteins levels assessment in culture containing DFMO. (A) Western blotting analysis of Cdk2, RhoA and c-Myc expression in different treatments containing DFMO; Semi-quantitative analysis of Cdk2 (B), RhoA (C) and c-Myc (D) in each group. (*P < 0.05, compared with the control group; # P < 0.05, compared with the DFMO group. n = 3) Data were analyzed by one-way ANOVA followed by SNK-test.

Although the effect of total ginsenosides on indomethacin-induced small intestinal mucosal injury in rats was not observed in this study, combining the results of the above related studies, we hypothesize that total ginsenosides also have similar pharmacological effects to ginseng herbs in this animal model. We will subsequently consider observing the effect of total ginsenosides on indomethacin-induced small intestinal mucosal injury in rats to enrich our understanding of the protective effect of ginseng on intestinal mucosa and the material basis of its pharmacological effects.

c-Myc, Cdk2 and RhoA are not only important regulators of cell cycle and cell proliferation, but also the targets of total

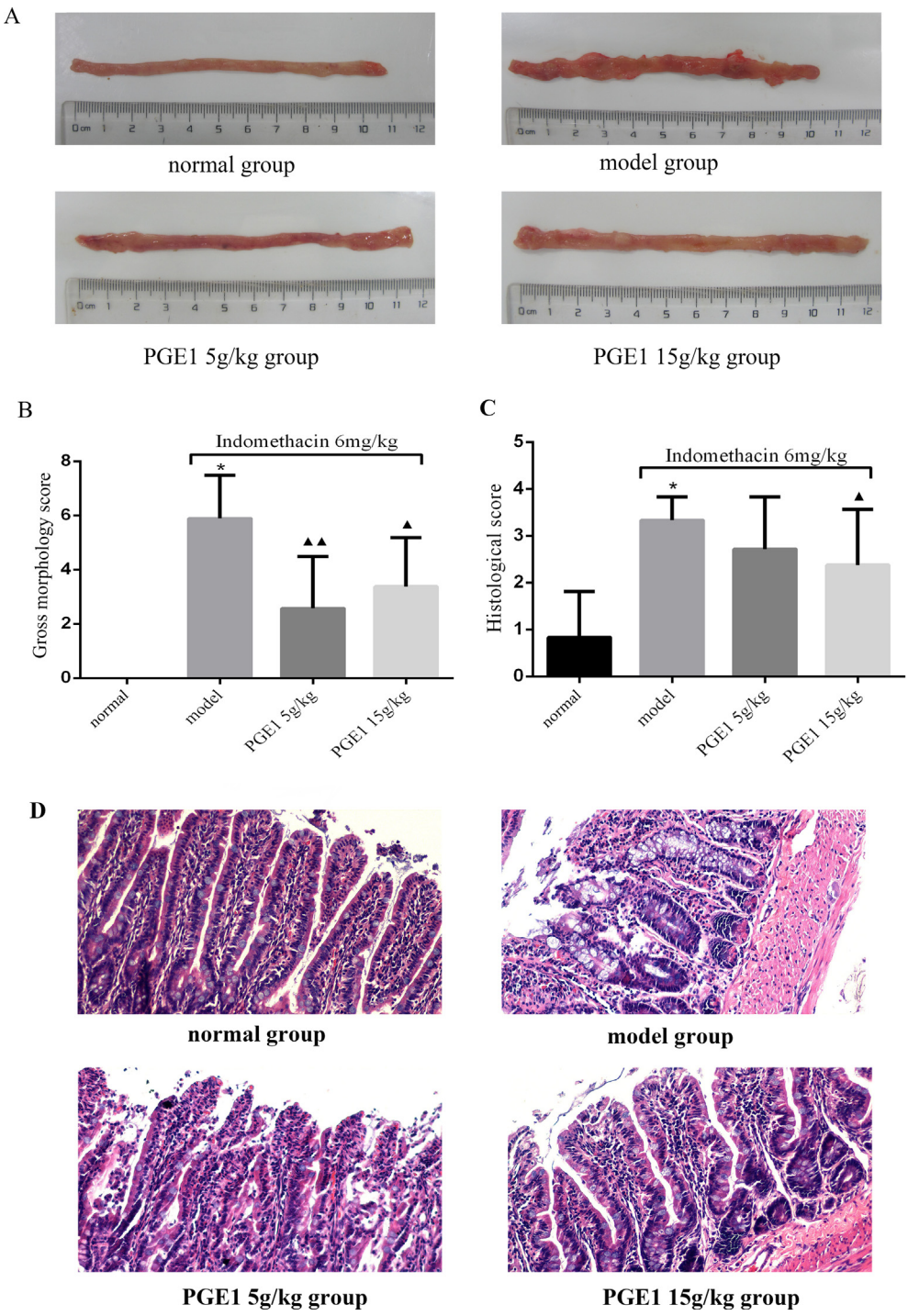

Fig. 6. Evaluation of small intestinal mucosal injury in rats. (A) Gross morphological manifestation of small intestinal mucosa in each group; (B) Gross morphology score of small intestinal mucosa in each group; (C) Histopathological score of small intestinal mucosal injury in each group; (D) Histopathological features of small intestinal mucosa in each group by H&E staining. (*P < 0.05 and **P < 0.01, compared with the normal group; ▲P < 0.05 and▲▲P < 0.01, compared with the model group. normal group n = 6, model group n = 9, PGE1 5 g/kg group n = 7 and PGE1 15 g/kg group n = 8.) Data were analyzed by Kruskal-Wallis H test followed by Mann-Whitney *U* test.

ginsenosides in promoting IEC-6 cell proliferation, which are regulated by polyamines. In the present study, PGE3 (total ginsenosides) promoted the proliferation of IEC-6 cells as well as reversed the cytostatic effect of DFMO on cell proliferation. What's more, PGE3 treatment resulted in decreasing percentage of IECs in G0/G1 phase as well as more cells in G2/M + S phase, which promoted the transition of IEC-6 cells from resting state to mitotic cell cycle. The results in the current study revealed that the expression level of cell proliferation-related proteins c-Myc, Cdk2 and RhoA were enhanced by the administration of PGE3. Besides, PGE3 could restore low expression of c-Myc, RhoA and Cdk2 induced by DFMO to the normal level. These findings suggested that total ginsenosides was beneficial to cell proliferation partially through regulating the related proteins c-Myc, Cdk2 and RhoA expression. We further detected the preventive effect of PGE1 (ginseng aqueous extracts) on intestinal mucosal injury, results indicated that PGE1

accelerated mucosal healing in vivo, possibly by promoting cell proliferation, which may provide valuable reference for exploring the protective mechanism of total ginsenosides on gastrointestinal mucosa in vivo.

Panax ginseng has been reported to have complex chemical components and a wide range of pharmacological effects with multi-target and multi-pathway characteristics. Ginsenosides and ginseng polysaccharides are responsible for the multiple pharmacological activities of *Panax ginseng* (In et al., 2017; Zhao et al., 2019). Our previous studies revealed that ginseng polysaccharides could promote the IEC-6 migration and the mechanism was related to its effect on Ca^{2+} related regulators in the polyamines signaling pathway. Ginseng polysaccharides could enhance the expression of TRPC1, Cav-1, PLC- γ1, RhoA and Rac1 proteins and the level of RhoA/TRPC1, Rac1/PLC- γ1, Cav-1/TRPC1 complex. Moreover, ginseng polysaccharides were also effective in alleviating indomethacin-induced intestinal mucosal injury and enhancing Ca^{2+} content in small intestinal mucosa of model rats (Zhu, 2020). We also observed the effect of total ginsenosides (50,100,200 mg/l) on the IEC-6 migration. The results showed that total ginsenosides (50,100 mg/l) had no significant impact on cell migration, however, high-dose total ginsenosides (200 mg/l) could promote cell migration, which suggested that the pharmacodynamic effect of total ginsenosides on promoting cell migration was comparatively weak, as compared with promoting cell proliferation. In addition, the expression level of c-Myc was upregulated by treatment with total ginsenosides during cell migration (Zhu, 2019). Thus, combined with our current work, ginseng polysaccharides tend to promote cell migration while total ginsenosides tend to promote cell proliferation, indicating that different ingredients from *Panax ginseng* may have different active effects. However, the effects of both total ginsenosides and ginseng polysaccharides on IEC-6 cells are associated with polyamines related regulators, and some of the targets are the same. The increase of RhoA protein expression occurs not only in the process of cell proliferation promoted by total ginsenosides, but also in the process of cell migration promoted by ginseng polysaccharides. Total ginsenosides could enhance c-Myc protein expression during both cell proliferation and cell migration. Gastrointestinal mucosal repair after injury is a complex process. *Panax ginseng* contains complex chemical components and a wide range of pharmacological effects with multi-target and multi-pathway characteristics (Shin et al., 2018; Kim, 2012; Wang, 2018; Zhao et al., 2020; Sung et al., 2020). The various components from *Panax ginseng* may have different active effects on IEC-6 cells, but polyamines related regulators are the common action targets of total ginsenosides and ginseng polysaccharides, which may also be one of the mechanisms and pharmacodynamic material bases for *Panax ginseng* to play a comprehensive role in the repair of gastrointestinal mucosal injury.

5. Conclusion

Our findings demonstrated that total ginsenosides could promote cell proliferation and regulate cell cycle via decreasing proportion of G0/G1 phase cells and increasing the proportion of S + G2/M phase cells, and upregulate cell proliferation-related proteins(c-Myc,RhoA,Cdk2) that are regulated by polyamines. What's more, total ginsenosides could also reverse the cytostatic effect of DFMO upon cell proliferation, cell cycle and c-Myc, RhoA, Cdk2 proteins expression. *Panax ginseng* had a positive effect on the mucosal repair in rats model of intestinal mucosal injury induced indomethacin. These findings are more likely to provide a fundamental novel insight into the underlying mechanisms and clinical therapeutic strategies of *Panax ginseng* in repairing intestinal mucosal injury.

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.

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References

An, M.Y., Lee, S.R., Hwang, H.J., Yoon, J.G., Lee, H.J., Cho, J.A., 2021. Antioxidant and Anti-Inflammatory Effects of Korean Black Ginseng Extract through ER Stress Pathway. Antioxidants (Basel). 10 (1), 62. https://doi.org/10.3390/antiox10010062.

Birkl, D., O'Leary, M.N., Quiros, M., Azcutia, V., Schaller, M., Reed, M., Nishio, H., Keeney, J., Neish, A.S., Lukacs, N.W., Parkos, C.A., Nusrat, A., 2019. Formyl peptide receptor 2 regulates monocyte recruitment to promote intestinal mucosal wound repair. FASEB J. 33 (12), 13632–13643. https://doi.org/10.1096/fj.201901163R.

Bretones, G., Delgado, M.D., Leon, J., 2015. Myc and cell cycle control. BBA 1849 (5), 506–516. https://doi.org/10.1016/j.bbagrm.2014.03.013.

Butt, A.J., McNeil, C.M., Musgrove, E.A., Sutherland, R.L., 2005. Downstream targets of growth factor and oestrogen signalling and endocrine resistance: the potential roles of c-Myc, cyclin D1 and cyclin E. Endocr. Relat. Cancer 12 (Suppl 1), S47–S59. https://doi.org/10.1677/erc.1.00993.

Chan, C.H., Lee, S.W., Li, C.F., Wang, J., Yang, W.L., Wu, C.Y., Wu, J., Nakayama, K.I., Kang, H.Y., Huang, H.Y., Hung, M.C., Pandolfi, P.P., Lin, H.K., 2010. Deciphering the transcriptional complex critical for RhoA gene expression and cancer metastasis. Nat. Cell Biol. 12 (5), 457–467. https://doi.org/10.1038/ncb2047.

Chelakkot, C., Ghim, J., Ryu, S.H., 2018. Mechanisms regulating intestinal barrier integrity and its pathological implications. Exp. Mol. Med. 50 (8), 1–9. https://doi.org/10.1038/s12276-018-0126-x.

Chiu, C.J., McArdle, A.H., Brown, R., Scott, H.J., Gurd, F.N., 1970. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. Arch. Surg. 101 (4), 478–483. https://doi.org/10.1001/archsurg.1970.01340280030009.

Farré, R., Fiorani, M., Abdu Rahiman, S., Matteoli, G., 2020. Intestinal Permeability, Inflammation and the Role of Nutrients. Nutrients. 12 (4), 1185. https://doi.org/10.3390/nu12041185.

Flemming, S., Luissint, A.C., Kusters, D.H.M., Raya-Sandino, A., Fan, S., Zhou, D.W., Hasegawa, M., Garcia-Hernandez, V., García, A.J., Parkos, C.A., Nusrat, A., Mostov, K., 2020. Desmocollin-2 promotes intestinal mucosal repair by controlling integrin-dependent cell adhesion and migration. Mol. Biol. Cell 31 (6), 407–418. https://doi.org/10.1091/mbc.E19-12-0692.

Gao, J.H., Guo, L.J., Huang, Z.Y., Rao, J.N., Tang, C.W., 2013. Roles of cellular polyamines in mucosal healing in the gastrointestinal tract. J. Physiol. Pharmacol. 64 (6), 681–693.

Grosheva, I., Zheng, D., Levy, M., Polansky, O., Lichtenstein, A., Golani, O., Dori-Bachash, M., Moresi, C., Shapiro, H., Del Mare-Roumani, S., Valdes-Mas, R., He, Y., Karbi, H., Chen, M., Harmelin, A., Straussman, R., Yissachar, N., Elinav, E., Geiger, B., 2020. High-Throughput Screen Identifies Host and Microbiota Regulators of Intestinal Barrier Function. Gastroenterology 159 (5), 1807–1823. https://doi.org/10.1053/j.gastro.2020.07.003.

Guo, H., Ray, R.M., Johnson, L.R., 2003. RhoA stimulates IEC-6 cell proliferation by increasing polyamine-dependent Cdk2 activity. Am. J. Physiol. Gastrointest. Liver Physiol. 285 (4), G704–G713. https://doi.org/10.1152/ajpgi.00044.2003.

Hiai, S., Oura, H., Hamanaka, H., Odaka, Y., 1975. A color reaction of panaxadiol with vanillin and sulfuric acid. Planta Med. 28 (2), 131–138. https://doi.org/10.1055/s-0028-1097841.

In, G., Ahn, N.G., Bae, B.S., Lee, M.W., Park, H.W., Jang, K.H., Cho, B.G., Han, C.K., Park, C.K., Kwak, Y.S., 2017. In situ analysis of chemical components induced by steaming between fresh ginseng, steamed ginseng, and red ginseng. J. Ginseng Res. 41 (3), 361–369. https://doi.org/10.1016/j.jgr.2016.07.004.

Kim, J.G., Islam, R., Cho, J.Y., Jeong, H., Cap, K.C., Park, Y., Hossain, A.J., Park, J.B., 2018. Regulation of RhoA GTPase and various transcription factors in the RhoA pathway. J. Cell. Physiol. 233 (9), 6381–6392. https://doi.org/10.1002/jcp.v233.910.1002/jcp.26487.

Kim, J.H., 2012. Cardiovascular Diseases and Panax ginseng: A Review on Molecular Mechanisms and Medical Applications. J. Ginseng Res. 36 (1), 16–26. https://doi.org/10.5142/jgr.2012.36.1.16.

Kim, J.H., Yi, Y.S., Kim, M.Y., Cho, J.Y., 2017. Role of ginsenosides, the main active components of Panax ginseng, in inflammatory responses and diseases. J. Ginseng Res. 41 (4), 435–443. https://doi.org/10.1016/j.jgr.2016.08.004.

Li, R.L., Mo, Q.Y., Wang, Z.P., Lin, C.Q., Chen, W.X., Chen, W.W., 2010a. Effects of Different Extract Parts from Rhizoma Atractylodis Macrocephalae and Radix Astragali on Proliferation of Intestinal Epithelial Cells. Traditional Chinese Drug Research and Clinical Pharmacology. 21 (2), 156–160 (in Chinese).

Li, R.L., Song, H.P., Wu, T.T., Tu, X.H., Lin, C.Q., Chen, W.W., 2015a. Effect of Methanol Extracts from Rhizoma Atractylodis Macrocephalae on Expression of Rho GTPaes and Myosin Ⅱ During Intestinal Epithelial Cell Migration. Traditional Chinese Drug Research and Clinical Pharmacology. 26 (6), 739–743 (in Chinese).

Li, R.L., Tao, Y.Z., Wen, P., Tu, X.H., Cai, J.Z., Chen, W.W., 2015b. Effect of Codonopsis and Glycyrrhiza glycoconjugates on migration and membrane potential of IEC-6 cells. Chinese Journal of Pharmacology and Toxicology. 29 (6), 917–923 (in Chinese).

Li, R.L., Tao, Y.Z., Zeng, D., Zhao, S.Q., Lin, C.Q., Chen, W.W., 2016. Effect of Codonopsis and Glycyrrhizae saccharide extracts on polyamine-dependent signaling pathway during cell migration in IEC-6. Chinese Pharmacological Bulletin. 32 (2), 245–251 (in Chinese).

Li, R.L., Xu, S.F., Chen, W.W., 2010b. Effects of Atractylodes macrocephala Monosaccharide Composition on Cytodifferentiation and Villin Expression of IEC-6 Cells in vitro. Journal of Chinese Medicinal Materials. 33 (6), 938–944 (in Chinese).

Liu, L., Li, L., Rao, J.N., Zou, T., Zhang, H.M., Boneva, D., Bernard, M.S., Wang, J.Y., 2005. Polyamine-modulated expression of c-myc plays a critical role in stimulation of normal intestinal epithelial cell proliferation. Am. J. Physiol. Cell Physiol. 288 (1), C89–C99. https://doi.org/10.1152/ajpcell.00326.2004.

Liu, L., Rao, J.N., Zou, T., Xiao, L., Smith, A., Zhuang, R., Turner, D.J., Wang, J.Y., 2012. Activation of Wnt3a signaling stimulates intestinal epithelial repair by promoting c-Myc-regulated gene expression. Am. J. Physiol. Cell Physiol. 302 (1), C277–C285. https://doi.org/10.1152/ajpcell.00341.2011.

Liu, L., Rao, J.N., Zou, T., Xiao, L., Wang, P.Y., Turner, D.J., Gorospe, M., Wang, J.Y., Tansey, W.P., 2009. Polyamines regulate c-Myc translation through Chk2-dependent HuR phosphorylation. Mol. Biol. Cell 20 (23), 4885–4898. https://doi.org/10.1091/mbc.e09-07-0550.

Mancuso, C., Santangelo, R., 2017. Panax ginseng and Panax quinquefolius: From pharmacology to toxicology. Food Chem. Toxicol. 107, 362–372. https://doi.org/10.1016/j.fct.2017.07.019.

McElrath, C., Espinosa, V., Lin, J.D., Peng, J., Sridhar, R., Dutta, O., Tseng, H.C., Smirnov, S.V., Risman, H., Sandoval, M.J., Davra, V., Chang, Y.J., Pollack, B.P., Birge, R.B., Galan, M., Rivera, A., Durbin, J.E., Kotenko, S.V., 2021. Critical role of interferons in gastrointestinal injury repair. Nat. Commun. 12 (1), 2624. https://doi.org/10.1038/s41467-021-22928-0.

Nian, L.Q., 2013. The study of the effect of Yiqi Jianpi herbs on stress ulcer in Rats and Polyamines. (in Chinese).

Odenwald, M.A., Turner, J.R., 2013. Intestinal permeability defects: is it time to treat? Clin Gastroenterol Hepatol. 11 (9), 1075–1083. https://doi.org/10.1016/j.cgh.2013.07.001.

Ohtani, K., DeGregori, J., Nevins, J.R., 1995. Regulation of the cyclin E gene by transcription factor E2F1. PNAS 92 (26), 12146–12150. https://doi.org/10.1073/pnas.92.26.12146.

Peng, C., Lei, Z.Q., 1997. Effect of ginsenosides on reinforcing Qi and strengthening spleen. Pharmacology and Clinics of Chinese Materia Medica. 13 (5), 17–19 (in Chinese).

Peng, C., Lei, Z.Q., 1999. Study on the Mechanism of Ginsenosides against Gastrointestinal Cell injury in Animals with spleen deficiency. Chinese Journal of Integrated Traditional and Western Medicine. 19 (S1), 54–56 (in Chinese).

Rao, J.N., Xiao, L., Wang, J.Y., 2020. Polyamines in Gut Epithelial Renewal and Barrier Function. Physiology (Bethesda). 35 (5), 328–337. https://doi.org/10.1152/physiol.00011.2020.

Ray, R.M., McCormack, S.A., Johnson, L.R., 2001. Polyamine depletion arrests growth of IEC-6 and Caco-2 cells by different mechanisms. Am. J. Physiol. Gastrointest. Liver Physiol. 281 (1), G37–G43. https://doi.org/10.1152/ajpgi.2001.281.1.G37.

Shi, Y., Zhu, H., Li, R., Wang, D., Zhu, Y., Hu, L., Chen, W., 2019. Effect of polysaccharides from Sijunzi decoction on Ca^{2+} related regulators during intestinal mucosal restitution. Phytomedicine 58, 152880. https://doi.org/10.1016/j.phymed.2019.152880.

Shi, Y.X., Li, R.L., Deng, J., Wang, D.X., Zhu, Y.P., Hu, L., Chen, W.W., 2018. The experimental model of intestinal epithelial cell growth established by real time cell analysis. Chinese Pharmacological Bulletin. 34 (02), 290–294 (in Chinese).

Shin, M.S., Song, J.H., Choi, P., Lee, J.H., Kim, S.Y., Shin, K.S., Ham, J., Kang, K.S., 2018. Stimulation of Innate Immune Function by Panax ginseng after Heat Processing. J. Agric. Food Chem. 66 (18), 4652–4659. https://doi.org/10.1021/acs.jafc.8b00152.

Song, H.P., Li, R.L., Chen, X., Wang, Y.Y., Cai, J.Z., Liu, J., Chen, W.W., 2014. Atractylodes macrocephala Koidz promotes intestinal epithelial restitution via the polyamine–voltage-gated K+ channel pathway. J. Ethnopharmacol. 152 (1), 163–172. https://doi.org/10.1016/j.jep.2013. 12.049.

Sung, W.S., Kang, H.R., Jung, C.Y., Park, S.S., Lee, S.H., Kim, E.J., 2020. Efficacy of Korean red ginseng (Panax ginseng) for middle-aged and moderate level of chronic fatigue patients: A randomized, double-blind, placebo-controlled trial. Complement Ther Med. 48, 102246. https://doi.org/10.1016/j.ctim.2019.102246.

Tu, X.H., Li, R.L., Deng, J., Cai, J.Z., Wu, T.T., Chen, W.W., 2016a. Effect of Sijunzi Decoction Polysaccharide on IEC-6 Cell Migration. Potassium Channel and Membrane Potential. Journal of Chinese Medicinal Materials. 39 (4), 856–862 (in Chinese).

Tu, X.H., Li, R.L., Deng, J., Zeng, D., Cai, J.Z., Chen, W.W., 2016b. Effects of Sijunzi Decoction polysaccharide on polyamine mediated calcium signaling pathway during intestinal epithelial cell migration. China Journal of Traditional Chinese Medicine and Pharmacy. 31 (5), 1665–1673 (in Chinese).

Wang, D.X., 2018. Experimental Studys on Prevention and Treatment Effects of Sijunzi Decoction on the Gastrointestinal Mucosal Injury by Influence Polyamines. (in Chinese).

Wang, D.X., Li, R.L., Zhu, Y.P., Shi, Y.X., Zhu, H.B., Hu, L., Chen, W.W., 2018a. The Mechanism of Sijunzi Decoction in Preventing Gastric Mucosal Injury from the Perspective of Polyamines and Cell Junction Proteins. Journal of Chinese Medicinal Materials. 41 (12), 176–184 (in Chinese).

Wang, W., Wang, S., Liu, J., Cai, E., Zhu, H., He, Z., Gao, Y., Li, P., Zhao, Y., 2018b. Sesquiterpenoids from the root of Panax Ginseng protect CCl_4-induced acute liver injury by anti-inflammatory and anti-oxidative capabilities in mice. Biomed. Pharmacother. 102, 412–419. https://doi.org/10.1016/j.biopha.2018.02.041.

Weiger, T.M., Hermann, A., 2014. Cell proliferation, potassium channels, polyamines and their interactions: a mini review. Amino Acids 46 (3), 681–688. https://doi.org/10.1007/s00726- 013-1536-7.

Wu, T.T., Li, R.L., Zeng, D., Hu, L., Shi, Y.X., Wang, D.X., Chen, W.W., 2017. Research of Atractylodes macrocephala Polysaccharides in Promoting Cell Migration and E-cadherin Expression Through Regulating Calcium Ions. Traditional Chinese Drug Research and Clinical Pharmacology. 28 (2), 145–150 (in Chinese).

Zeng, D., Hu, C., Li, R.L., Lin, C.Q., Cai, J.Z., Wu, T.T., Sui, J.J., Lu, W.B., Chen, W.W., 2018. Polysaccharide extracts of Astragalus membranaceus and Atractylodes macrocephala promote intestinal epithelial cell migration by activating the polyamine-mediated K^+ channel. Chin J Nat Med. 16 (9), 674–682. https://doi.org/10.1016/S1875-5364(18)30107-9.

Zeng, D., Li, R.L., Wu, T.T., Wang, D.X., Shi, Y.X., Chen, W.W., 2017. Effects of polysaccharides from Astragalus membranaceuson polyamines mediated signaling pathway during intestinal epithelial cell (IEC-6) migration. China Journal of Traditional Chinese Medicine and Pharmacy. 32 (8), 3447–3452 (in Chinese).

Zhang, J., Liu, S., Ye, Q., Pan, J., 2019. Transcriptional inhibition by CDK7/9 inhibitor SNS-032 abrogates oncogene addiction and reduces liver metastasis in uveal melanoma. Mol Cancer. 18 (1), 140. https://doi.org/10.1186/s12943-019-1070-7.

Zhang, Y., Cai, J.Z., Xiao, L., Chung, H.K., Ma, X.X., Chen, L.L., Rao, J.N., Wang, J.Y., 2020. RNA-binding protein HuR regulates translation of vitamin D receptor modulating rapid epithelial restitution after wounding. Am. J. Physiol. Cell Physiol. 319 (1), C208–C217. https://doi.org/10.1152/ajpcell.00009.2020.

Zhao, B., Lv, C., Lu, J., 2019. Natural occurring polysaccharides from Panax ginseng C. A. Meyer: A review of isolation, structures, and bioactivities. Int. J. Biol. Macromol. 133, 324–336. https://doi.org/10.1016/j.ijbiomac.2019.03.229.

Zhao, B., Wang, X., Liu, H., Lv, C., Lu, J., 2020. Structural characterization and antioxidant activity of oligosaccharides from Panax ginseng C. A. Meyer. Int J Biol Macromol. 150, 737–745. https://doi.org/10.1016/j.ijbiomac.2020.02.016.

Zhu, Y.P., 2019. The Research on the repair mechanism of gastrointestinal mucosal injury of Astragali and Ginseng from the perspective of cell proliferation and adhesion. (in Chinese).

Zhu, F., Qian, J.M., Pan, G.Z., 1998. The establishment of TNBS-induced experimental colitis. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 20 (4), 271–278.

Zhu, H.B., 2020. Studies on the Effect of Panax ginseng Polysaccharide on Ca^{2+} Related Regulator during Intestinal Epithelial Cell Migration. (in Chinese).