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ORIGINAL ARTICLE

Pachymic acid alleviates TNBS-induced intestine mucosal injury in mice

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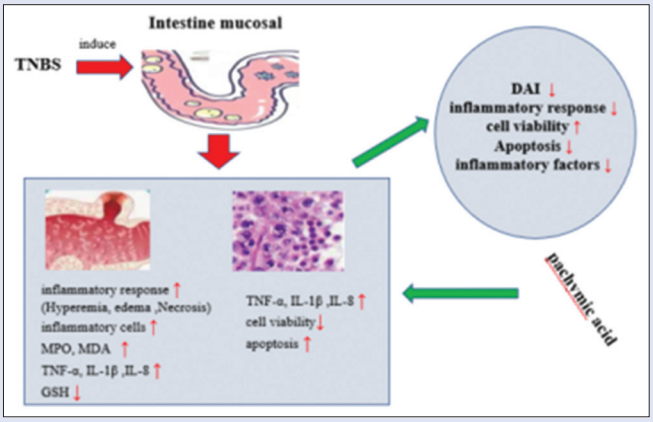
ABSTRACT

Objective: Pachyman might reduce the intestinal dysfunction and inflammation. Therefore, in this study, we aimed to explore the effect of pachymic acid on repairing the intestine mucosal injury in mice. **Materials and Methods:** Male BALB/c mice were randomly divided into six groups: control group, trinitrobenzenesulfonic acid (TNBS)-induced group, pachymic acid (50, 100, and 200 mg/kg) groups, and mesalazine group. In addition to the mice in the control group, all mice in other groups were induced by TNBS, an inducer of colitis, to establish the intestine mucosal injury model. To study the effect of pachymic acid on the intestine mucosal injury in mice, we assessed the disease activity index (DAI) and the morphology colon specimen in mice. Histopathological examination was also used to access the effects of different concentrations of pachymic acid on the intestinal mucosal injury in mice. Enzyme-linked immunosorbent assay (ELISA) tests were performed to detect the level of glutathione (GSH), myeloperoxidase (MPO), (MDA), and pro-inflammatory factors (tumor necrosis factor-α [TNF-α], interleukin (IL)-1 β, and IL-8) to evaluate the effect of pachymic acid on the biomarkers of oxidative stress and pro-inflammatory factors. At the *in vitro* level, the human colon carcinoma cells (Caco-2) were induced by TNBS to establish the *in vitro* intestinal mucosal injury model. After treatment with the pachymic acid, the cell viability was examined by methyl thiazolyl tetrazolium assay, and the cell apoptosis was detected by flow cytometric analysis. The expression levels of apoptosis-related proteins were analyzed by Western blot analysis. Meanwhile, the secretion level of TNF-α, IL-1 β, and IL-8 in the supernatant of Caco-2 cells was detected by ELISA kits. **Results:** Pachymic acid was found to reduce the DAI of intestine mucosal injury in treated mice and improved the hyperemia, edema, and necrosis in intestinal mucosal tissue. Pachymic acid decreased the secretion levels of pro-inflammatory factors TNF-α, IL-1 β, and IL-8, as well as the oxidative stress markers, MPO and MDA. In contrast, the serum levels of GSH were found to be increased. After being challenged by TNBS, the cell viability of Caco-2 cells was inhibited, while after treated with pachymic acid, inhibitory effect was partially blocked. Similarly, the TNBS-induced Caco-2 cells' apoptosis was abolished by pachymic acid. **Conclusion:** Pachymic acid repaired the intestine mucosal injury and attenuated inflammatory response in experimental mice. It provides an alternative therapeutic strategy for intestine mucosal injury and highlights the protective effects of traditional Chinese medicine in these diseases.

Key words: Chinese medicine, inflammatory response, intestinal mucosal injury, pachymic acid, protective effect, repairing effect

SUMMARY

- In summary, our exploration provides the obvious evidence that pachymic acid has the potential effect of repairing the intestinal mucosal injury and that it attenuates inflammatory response in mice. This study demonstrated an experimental basis for further clinical trials with pachymic acid in intestinal mucosal injury.



Abbreviations used: TNBS: Trinitrobenzenesulfonic acid; DAI: Disease activity index; GSH: Glutathione; MPO: Myeloperoxidase; MDA: Malondialdehyde; TNF-α: Tumor necrosis factor-α; IL-1 β: Interleukin-1 β; IL-8: Interleukin-8; MTT: Methyl thiazolyl tetrazolium.

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INTRODUCTION

Intestinal mucosa is an important barrier with the function of preventing harmful substances and pathogens and maintaining the stability of the internal environment. The intestinal mucosal injury might help in the translocation of bacteria and various endotoxins.[1] The major clinical manifestations of intestinal mucosal injury are diarrhea, abdominal pain, constipation, bloody purulent stool, tenesmus, weight loss, and fatigue.[2] Therefore, repairing the damaged intestinal mucosa is an important step to improve intestinal mucosal injury and homeostasis of the physiological system.

The intestinal mucosal injury can be caused due to various factors but is frequently seen in inflammatory bowel disease, such as Crohn's disease

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and ulcerative colitis[3] where the development is related to genetic predispositions and the specific environmental factors. The medication management for intestinal mucosal injury includes 5-aminosalicylic acid drugs, steroids, and immunosuppressants. In addition, colectomy is performed on patients with refractory or colonic neoplasia.[4] With the increasing number of patients with intestinal mucosal injury and the improved recognition of the related diseases, many new treatment strategies are being provided to the patients. Numerous studies have revealed that traditional Chinese medicine and their derived active constituents can reduce intestinal inflammation and promote wound healing through multiple mechanisms. These phytoconstituents include quercetin, resveratrol, curcumin, berberine, silybin, curcumin, and shikimic acid.[5] According to the literature, pachyman might reduce the intestinal dysfunction and inflammation; therefore, we aimed to investigate the effect of the other primary component of *Poria cocos*, namely pachymic acid [Figure 1 shows the chemical structure of pachymic acid] on intestinal mucosal injury.

P. cocos is the dried sclerotium of Polyporaceae fungi, consisting of polysaccharides, triterpenoids, fatty acids, sterols, and enzymes. It exhibits antitumor, anti-hepatitis, immunomodulation, anti-inflammation, antioxidation, antiaging, antidiabetes, and antihemorrhagic properties.[6] Pachymic acid is used to treat patients with insomnia.[7] In sepsis-induced acute kidney injury model, pachymic acid suppresses the inflammation and has the antioxidant effect through activating the Nrf2/HO-1 pathway in Sprague–Dawley rats.[8] Pachymic acid inhibited tumorigenesis of gastric cancer,[9,10] nasopharyngeal cancer,[11] gallbladder cancer,[12] and other diseases.

Pachyman is a Traditional Chinese Medicine which alleviates intestinal dysfunction and inflammation, meanwhile pachymic acid is the effective active pharmaceutical ingredients of pachyman. In this study, we established the colitis model to investigate its effect on repairing the intestinal mucosal injury and the underlying mechanism of action.

Although pachymic acid shows strong effects on gastric disease, the effects of pachymic acid on colitis remain elusive. In this study, the effects of pachymic acid on colitis were investigated both under *in vivo* and *in vitro* conditions. Moreover, the mechanism of gastric repairing was also investigated. The results of this study might provide a theological basis for the industry in the application of pachymic acid in gastric diseases.

MATERIALS AND METHODS

Materials

Pachymic acid was purchased from the Shanghai Yuanye Bio-Technology company. Trinitrobenzenesulfonic acid (TNBS), chloral hydrate, polyethylene catheter, and 50% ethanol were provided by Nanjing Zelang Medical Technological Co., Ltd (Nanjing, China). Next, 4% paraformaldehyde was provided by Shrbio (Nanjing, China). K_2-EDTA anticoagulant tubes were provided by Becton, Dickinson and Company. Myeloperoxidase (MPO), malondialdehyde (MDA), reduced glutathione (GSH), tumor necrosis factor (TNF)-α, interleukin (IL)-1 β, and IL-8 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Nanjing Fcmacs Biotech Co., Ltd.

Animals

There occur hormonal changes during estrus in female mice. To avoid the hormone disturbance, the male mice were accepted in this study. Male BALB/c mice (6–8 weeks, 18–22 g) were provided by the animal center of Zhejiang hospital and were housed in the animal center of Zhejiang hospital with conventional free eating for 1 week. The room temperature and relative humidity were maintained at 20°C ± 2°C and 45%–55%, respectively. All studies were performed according to the guidelines of experimental animals and were approved by the Animal Experiment Ethics Review Committee of Zhejiang Hospital (ZJLL-20191206).

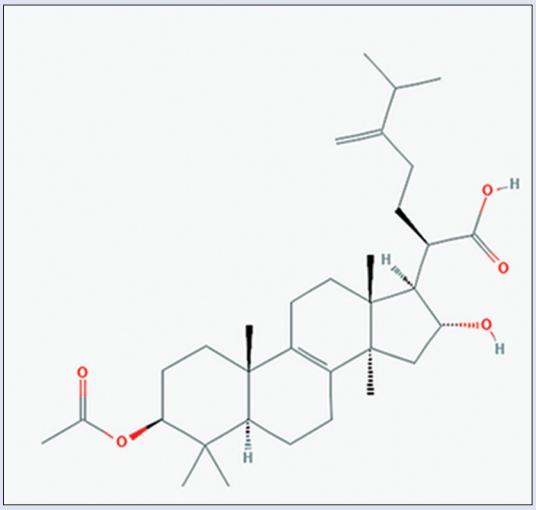

Figure 1: The chemical structure of pachymic acid

Colitis model

To induce colitis in the experimental mice, animals (n = 30) were anesthetized with 10% chloral hydrate and a polyethylene catheter (1 mm in outer diameter) was inserted into the colon (4 cm from the anus).[13] Then, 2,4,6-TNBS (TNBS, 100 mg/kg dosage dissolved in 0.25 mL of 50% ethanol) was administered through the catheter. After the administration of TNBS, mice were held in a headfirst position for 3 min to prevent the leaking out of the solution.

Pachymic acid administration

Mice were randomly divided into the following six groups (n = 6): control group, TNBS-induced group, pachymic acid (50, 100, 200 mg/kg) groups, and mesalazine group. Once the colitis model was successfully established, the mice in mesalazine and pachymic acid groups were administered with mesalazine and pachymic acid. Mice in the control and TNBS-induced groups were treated with saline solution (once daily for 1 week). The doses of pachymic acid were selected based on a previous study.[14] The dose range we selected has been demonstrated to be safe for the mice. After 12 days of treatment, the mice were anesthetized with 10% chloral hydrate, and the eye blood samples were collected into the K_2-EDTA anticoagulant tubes. Finally, the mice were sacrificed, and the colonic tissues were quickly removed and rinsed with ice-cold PBS.

Measurement of disease activity index

The Modified Mayo Score System was used as a reference standard.[15,16] We measured the Disease Activity Index (DAI) based on the frequency, viscosity of tools, and accompanying symptoms. DAI was scored as 0–1 where 0 indicates normal stool without any bleeding, 2 indicates thin stool mixed with blood with a frequency of 3 times, 3 indicates the frequency of diarrhea as more than 3–4 times and blood mixed in stool for most of the times, and 4 indicates diarrhea over 5 times and with continuous bleeding. In addition to the DAI assessment criteria, we considered histopathological analysis.

Enzyme-linked immunosorbent assay

After 12 days, blood samples were collected in K_2-EDTA anticoagulant tubes and serum was separated by centrifuging for 10 min at 3000 rpm. Then, the levels of MPO, MDA, GSH, TNF-α, IL-1 β, and IL-8 were analyzed in serum using ELISA kits. ELISA tests were performed to evaluate the level of secretion of these factors in the supernatant from the cultured Caco-2 cells. All methods were referred to the manual from the ELISA kit.

Histopathological analysis

We performed hematoxylin and eosin (H and E) staining for the histopathological analysis. The colon specimens were collected. From this, 4 μm specimens were fixed in 4% paraformaldehyde (Shrbio, Nanjing, China), dehydrated, and finally, were embedded in paraffin for analysis (Leica, Shanghai, China). Then, specimens were staining using hematoxylin for 5 min to stain the nucleus and with eosin for 2 min to stain the cytoplasm. The histological images of the specimens were acquired (Olympus, Beijing, China) at the magnification of ×200. The staining results revealed that the blue-stained area was the nucleus and the red-stained area was the cytoplasm.

Cell culture and treatments

Human colon carcinoma cells (Caco-2 cells) were purchased from American Type Culture Collection and routinely cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin (Thermo Fisher, Shanghai, China) and in a humidified atmosphere with 5% CO_2 at 37°C. The Caco-2 cells were inoculated into six different plates (Corning Incorporated, USA) for further investigation. Except the control group, we set another five groups for the TNBS-treated group and pachymic acid (5, 10, and 20 μM) groups. The doses of pachymic acid we selected were based on a previous study conducted on gastric cancer cells (SGC-7901 cells), in which pachymic acid was used in 20, 40, and 80 μM concentration.[10] However, in another study conducted on WI-38 cells, the doses of pachymic acid selected were 1, 2, 4, 8, and 16 μM.[17] In this study, before treating Caco-2 cells with pachymic acid, cells in the pachymic acid group were challenged with 200 μg/mL TNBS for 24 h.

Methyl thiazolyl tetrazolium assay

Caco-2 cells were inoculated into 96-well plates (8–10 × 10^3 cells/well) after incubation with different concentrations of pachymic acid (0, 5, 10, and 20 μM) for 24 h. Then, we used the MTT assay kit (Thermo Fisher, Shanghai, China) to detect the cell viability. Briefly, 10 μL MTT solution was added to each well. After incubation for another 1 h (at 37°C), the optical density (OD) of each well was measured at 450 nm by a microplate reader (Molecular Devices, Shanghai, China). The percentages of the OD values of the samples to a blank control were recorded. The values for cell viability = (treatments − blank)/(control − blank).

Flow cytometric analysis

After incubating the cells with pachymic acid and mesalazine for 24 h, the cells were collected for apoptosis analysis. The Apoptosis Detection Kit was purchased from BD company (Biosciences, USA) and the experiment was conducted based on the manufacturer's instructions;[18] finally, the cells were *t* analyzed by flow cytometry (FACScan, BD Biosciences).

Western blot assay

After incubating the cells with pachymic acid and mesalazine for 24 h,[19] Western blot analysis was performed on the proteins isolated from the Caco-2. The antibodies were provided by Abcam company (Shanghai, China) which are as follows: anti-caspase-3, anti-caspase-9, anti-Bax, anti-Bcl-2, and anti-β-actin.

Statistical analyses

All experiments were repeated thrice, and the data were presented as the mean ± standard deviation. The data were analyzed using SPSS software version 18.0 (SPSS, Chicago, IL, USA). An unpaired Student's *t*-test was performed to compare the differences between the two groups. Furthermore, a one-way analysis of variance was performed followed by Bonferroni multiple comparison test to identify the differences between two or more groups. $P < 0.05$ was considered statistically significant.

RESULTS

The protective function of pachymic acid on repairing the intestinal mucosal injury in mice

According to the Modified Mayo index, the DAI score in TNBS was 4 when compared with the control group. This suggests that TNBS severely damaged the intestinal mucosa ($P < 0.001$) [Figure 2a]. After administration with pachymic acid and mesalazine, the scores of DAI were decreased, especially in 200 mg/kg pachymic acid group (100 mg/kg pachymic acid group: $P < 0.05$; 200 mg/kg pachymic acid group and mesalazine group: $P < 0.01$; compared to TNBS group). Figure 2b shows the morphological analysis of intestinal mucosa. This result shows that compared with the control group, the morphological characteristics of colon specimens in TNBS-treated group revealed that hyperemia, edema, necrosis, and the length were shortened. H and E staining demonstrated that the tissues in TNBS group were full of a large number of inflammatory cells and the cell boundaries were unclear [Figure 2c]. The histopathological score suggested that the colon specimens in TNBS-treated group revealed severe damage to the intestinal mucosa ($P < 0.01$, compared to control group) and after treatment with pachymic acid and mesalazine, the scores decreased [Figure 2d]. With the increase in the concentration of pachymic acid, the improvement was more significant. In theory, 200 mg/kg pachymic acid demonstrated better effect than that of mesalazine (100 mg/kg pachymic acid group: $P < 0.05$; 200 mg/kg pachymic acid group and mesalazine group: $P < 0.01$; compared to TNBS group).

The effect of pachymic acid on the inflammatory factors and oxidative stress biomarkers in the intestine mucosal injury

To validate the function of pachymic acid on the inflammatory factors and oxidative stress biomarkers, ELISA tests were performed to detect the level of secretion of MPO, MDA, GSH, TNF-α, IL-1 β, and IL-8. Among these factors, MPO represents the function and activity of neutrophilic polymorphonuclear leukocytes. In the case of inflammatory conditions, the activity and the protein levels of MPO increased. It could act as a potential independent prognostic biomarker for inflammation.[20] MDA is one of the most important products of membrane lipid peroxidation, which indicates tissue injury and has a pro-inflammatory effect.[21] GSH combined with glutamate, cysteine, and glycine has the ability to protect the cells and organisms against oxidative stress. It is a powerful antioxidant and a detoxifying agent.[22] In this study, the expression of MPO and MDA was increased in TNBS-treated group ($P < 0.01$, compared to control group), whereas their expression decreased after the treatment of cells with pachymic acid and mesalazine (all in 200 mg/kg pachymic acid group and mesalazine group, $P < 0.01$; MDA: 200 mg/kg pachymic acid group, $P < 0.05$; MPO: 50 mg/kg pachymic acid group, $P < 0.05$; 100 mg/kg pachymic acid group, $P < 0.01$; compared to TNBS

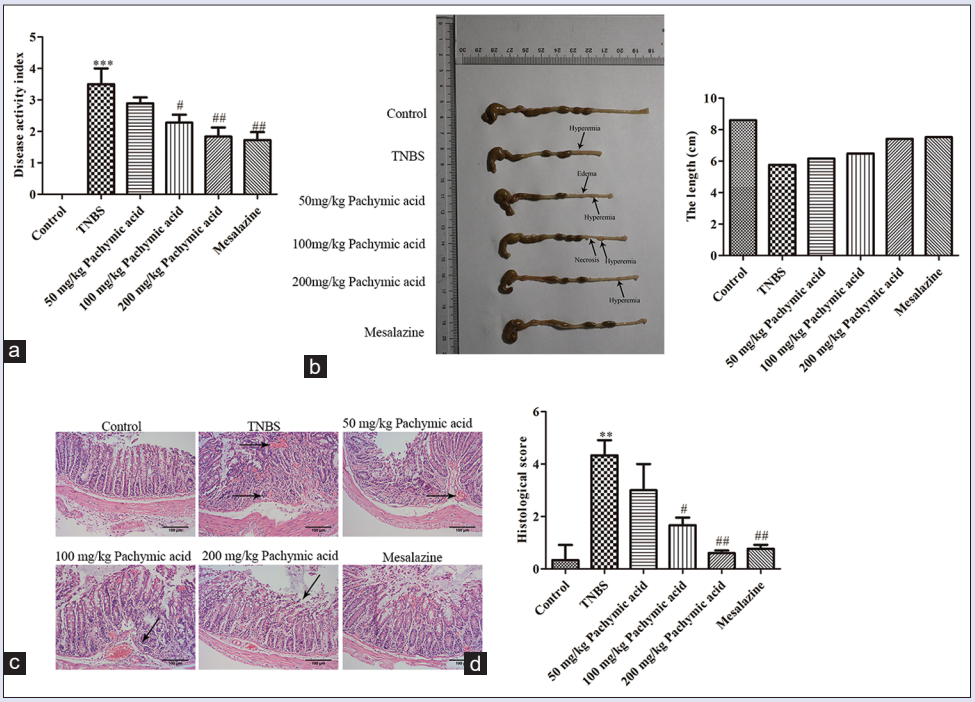

Figure 2: The function of pachymic acid in repairing the intestine mucosal injury in mice (a) The measurement of disease activity index. (b) Morphological examination of colon specimens (hyperemia, edema, necrosis, and length changes). (c) The assays of H and E staining (Magnificence × 200). (d) The detection of histopathological score (**P < 0.01 and ***P < 0.001, compared to control group, $^{\#}P$ < 0.05 and $^{\#\#}P$ < 0.01, compared to TNBS group). All the data were presented as mean ± standard deviation

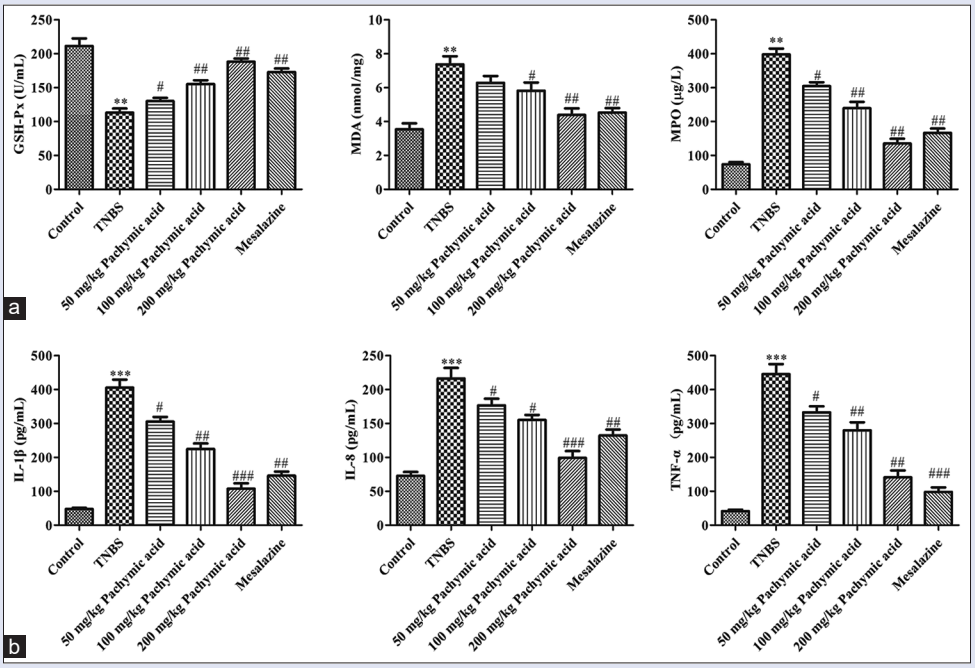

Figure 3: The effect of pachymic acid on regulating the inflammatory factors and oxidative stress biomarkers (a) The enzyme-linked immunosorbent assay for oxidative stress biomarkers, including myeloperoxidase, malondialdehyde, and glutathione. (b) The enzyme-linked immunosorbent assay for inflammatory factors, including TNF-α, IL-1 β, and IL-8.(**P < 0.01, *** P < 0.001, compared to control group, $^{\#}P$ < 0.05 and $^{\#\#}P$ < 0.01, $^{\#\#\#}P$ < 0.001, compared to TBNS group). All the data were presented as mean ± standard deviation

group). On the contrary, the expression of GSH was upregulated after treatment with TNBS (P < 0.01, compared to control group), whereas its expression decreased with the administration of pachymic acid and mesalazine (50 mg/kg pachymic acid group: P < 0.05; 100 and 200 mg/kg pachymic acid group, mesalazine P < 0.01; compared to TNBS group) [Figure 3a]. TNF-α, IL-1 β, and IL-8 are pro-inflammatory

factors. In this study, all the aforementioned parameters were increased in TNBS-treated group ($P < 0.01$, compared to control group), whereas pachymic acid and mesalazine decreased their levels (IL-1 β: 50 mg/kg pachymic acid group: $P < 0.05$; 100 and 200 mg/kg pachymic acid group, mesalazine $P < 0.01$; compared to TNBS group; IL-8: 50 and 100 mg/kg pachymic acid group: $P < 0.05$; 200 mg/kg pachymic acid group, $P < 0.05$; mesalazine $P < 0.01$; compared to TNBS group; TNF-α: 50 mg/kg pachymic acid group: $P < 0.05$; 100 and 200 mg/kg pachymic acid group, mesalazine $P < 0.01$; compared to TNBS group) [Figure 3b].

The effects of pachymic acid on the cell viability and apoptosis in the trinitrobenzenesulfonic acid-treated Caco-2 cells

MTT assay was performed to validate the effect of pachymic acid on the viability of Caco-2 cells. According to the results, TNBS significantly reduced the viability of Caco-2 cells ($P < 0.01$, compared to control group), whereas pachymic acid counteracted this effect, which was dose dependent. The effect was noticeable, especially for the high concentration of pachymic acid (10 μM: $P < 0.05$; 20 μM: $P < 0.01$; compared to TNBS group) (20 μM) [Figure 4a]. The apoptosis of the TNBS-treated Caco-2 cells was detected by flow cytometry analysis. TNBS significantly promoted the apoptosis of Caco-2 cells ($P < 0.001$, compared to control group). However, both pachymic acid and mesalazine inhibited the apoptosis of Caco-2 cells, and the inhibitory effect was positively correlated with the concentration of pachymic acid (10 μM: $P < 0.05$; 20 μM and mesalazine: $P < 0.01$; compared to TNBS group) [Figure 4b]. The results of Western blot analysis indicated that TNBS induced the increase protein expression levels of Bax and cleaved caspase 3 and 9 (Bax: $P < 0.01$; cleaved caspase 3 and 9: $P < 0.05$; compared to control group) and decreased the protein expression level of Bcl-2 (Bcl-2: $P < 0.01$, compared to control group). On the contrary, pachymic acid and mesalazine upregulated the expression levels of Bcl-2 (10 μM and mesalazine: $P < 0.05$; 20 μM: $P < 0.01$; compared to TNBS group) and downregulated the expression of Bax (5, 10, and 20 μM and mesalazine: $P < 0.01$; compared to TNBS group), cleaved caspase 3 (5 and 10 μM: $P < 0.01$; 20 μM and mesalazine: $P < 0.001$; compared to TNBS group), and cleaved caspase 9 (5 and 10 μM: $P < 0.01$; 20 μM and mesalazine: $P < 0.001$; compared to TNBS group) [Figure 4c].

The effects of pachymic acid on the inflammatory factors in the trinitrobenzenesulfonic acid-treated Caco-2 cells

As shown in Figure 5, compared with the cells in TNBS group, the levels of TNF-α, IL-β, and IL-8 in pachymic acid-treated groups were decreased, which was dose dependent (TNF-α and IL-1 β: 10 μM: $P < 0.05$; 20 μM and mesalazine group: $P < 0.01$; compared to TNBS group; IL-8: 5 μM, $P < 0.01$; 10 μM: $P < 0.01$; 20 μM and mesalazine group: $P < 0.005$; compared to the TNBS group).

DISCUSSION

Intestinal disorders are primarily caused due to the destruction of the normal structure and function of intestinal mucosa. Intestinal mucosal injury is considered to be one of the clinical features of Crohn's disease and ulcerative colitis.[23,24] The mechanism is related to the destruction of intestinal mechanical barrier and normal intestinal flora, leading to immune dysfunction.[25] Mesalazine is a 5-aminosalicylic acid (5-aminosalicylate) compound, and it is the most common medication available for ulcerative colitis, ulcerative proctitis, and Crohn's disease which has a significant inhibitory effect on the intestinal inflammation. Mesalazine inhibits the synthesis of prostaglandins, oxygen free radicals, and platelet activity factor. It can also reduce the release of prostaglandin E2 in colonic mucosa, and it inhibits the activity of lipase and the production of leukotriene B4 and leukotriene.[26] Given that mesalazine causes side effects in the digestive tract, it is necessary to

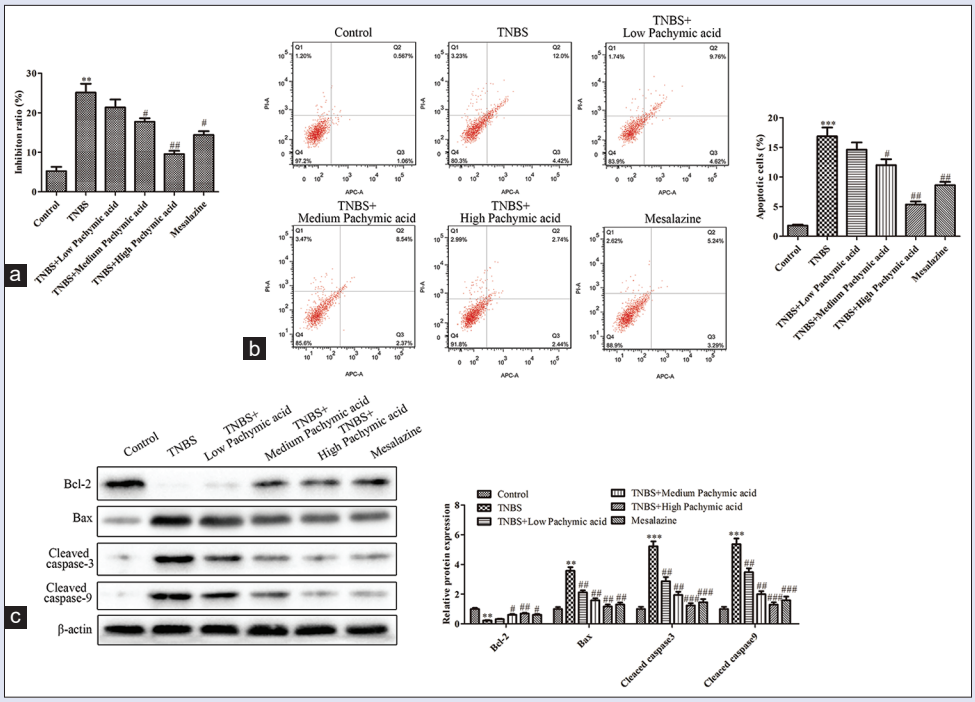

Figure 4: The effects of pachymic acid on cell viability and apoptosis in Caco-2 cells (a) Methyl thiazolyl tetrazolium assays for cell viability. (b) Flow cytometry analysis for apoptosis. (c) Western blot assay for the expression level of apoptosis-related proteins.(**$P < 0.01$, ***$P < 0.001$, compared to control group, #$P < 0.05$ and ##$P < 0.01$, ###$P < 0.001$, compared to TBNS group). All the data were presented as mean ± standard deviation

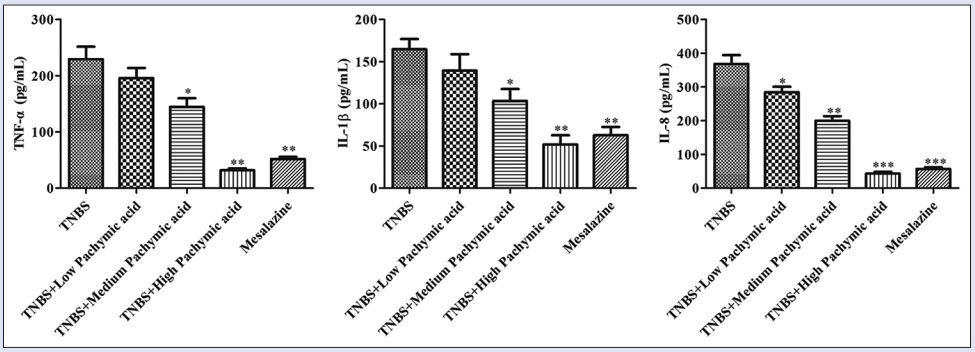

Figure 5: The effects of pachymic acid on the inflammatory factors in the trinitrobenzenesulfonic acid-treated Caco-2. The enzyme-linked immunosorbent assay for inflammatory factors, Tumor necrosis factor-α, interleukin-1 β, interleukin-8. Asterisks indicated significant differences from the control (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared to TBNS group). All the data were presented as mean ± standard deviation

develop new therapeutic drugs. In addition, mesalazine might act as a positive comparator to assess the function of pachymic acid in repairing the intestinal mucosal injury.

Pachymic acid is extracted from *P. cocos*. According to the traditional Chinese medicine, *P. cocos* belongs to the medicine that might alleviate the invasion of pathogenic dampness in human body and promote diuresis. *P. cocos* is widely used to treat the patients with edema, scanty urination, difficult urination, diarrhea. Modern pharmacology suggests that *P. cocos* can be used as a diuretic, sedative, antitumor, and an antidiabetic agent. It increases myocardial contractility, protects liver, reduces gastric juice secretion, and retards the formation of gastric ulcer. In addition, pachymic acid alleviates lung injury,[27] pancreatic cancer,[28] and lung cancer.[29] However, the effect of pachymic acid on the intestinal mucosal injury has not studied yet. Therefore, in this study, we demonstrated the protective effect of pachymic acid on repairing intestinal mucosal injury induced by TNBS. TNBS is a classic skin contactor that can be used to induce colitis.[30] Pachymic acid improved colitis by reducing inflammatory cytokines, increasing GSH levels, and inhibiting cellular apoptosis. In TNBS-treated group, the mice revealed that high DAI and their colon specimens were characterized with hyperemia, edema, necrosis, and a short colon length. H and E staining was performed, which revealed that the TNBS-treated colon samples were invaded with inflammatory cells and that the cell boundaries were unclear. In contrast, treatment with pachymic acid improved these inflammatory lesions. Our results show that the inflammation is negatively related to the concentration of pachymic acid content. All the effects due to pachymic acid were seen in a dose-dependent manner. In addition, 200 mg/kg pachymic acid exhibited a better therapeutic effect than that of the positive drug, mesalazine. Inflammation and oxidative stress can be extensively involved in the pathogenesis of intestinal mucosal injury.[31] In this study, pachymic acid decreased the secretion of MPO, MDA, TNF-α, IL-1 β, and IL-8, and increased the secretion of GSH in the serum of the mice with intestinal mucosal injury. GSH is a reduced form of GSH, and an increase in the level of GSH represents lowered oxidative stress. We examined the effects of pachymic acid on Caco-2 cells. Caco-2 cells are human cloned colon adenocarcinoma cells, with microvilli and other structures. These cells contain enzymes that related to the function of small intestinal brush-shaped margin epithelium. Due to the similarity to the differentiated small intestinal epithelial cells in structure and function, Caco-2 cells can be used to mimic intestinal transport under *in vivo* conditions.[32] Our investigation corroborated that pachymic acid might increase cell viability and inhibit cellular apoptosis. In addition, pachymic acid also decreased the secretion of TNF-α, IL-1 β, and IL-8.

CONCLUSION

Our study further demonstrated that pachymic acid significantly attenuated the inflammatory response in intestinal mucosal injury and decreased the levels of inflammatory mediators. Furthermore, pachymic acid promoted cell viability and inhibited apoptosis of TNBS-treated Caco-2 cells. Pachymic acid exhibited protective effects against intestinal mucosal injury via regulation of inflammation. These results show the effective role of traditional Chinese medicine. It provided a new perspective to explore another therapeutic effect that could be tested in the clinical setting in future.

Ethics approval and consent to participate

The experimental protocol was established according to the ethical guidelines of the Helsinki Declaration and was approved by the animal experiment ethics review committee of Zhejiang hospital (ZJLL-20191206).

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Nil.

Conflicts of interest

There are no conflicts of interest.

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