Open Journal of Clinical & Medical Images

www.jclinmedimages.org

ISSN: 2833-2725

Research Article

Open Access, Volume 5

Short report of ulcerative colitis rodent model treatment with taper up- off protocol of opium tincture revealed an expression alteration in wound healing genes

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Received: Jul 14, 2025

Accepted: Aug 05, 2025

Published: Aug 11, 2025

Archived: www.jclinmedimages.org

Keywords: Ulcerative colitis; Opium tincture; Taper up-off; Wound healing; Quantitative real-time PCR.

Abstract

Ulcerative Colitis (UC) is a chronic inflammatory bowel disease affecting the colon mucosa. The origin of UC is not completely clarified, but genetic and autoimmune reactions causing intestinal inflammation and injury were considered the main pathological manifestations. UC-caused injury is a risk factor for colon cancer. Inflammatory response inhibition, along with wound healing of colonic epithelial cells, is critical for UC treatment. The present short report examines the effects of opium tincture on wound-healing molecular pathways.

The rat model of UC was produced using Dextran Sulfate Sodium (DSS)-Induced acute colitis. The UC model rats were treated with taper up-off of opium tincture protocol called Dezhakam-Step-Time (DST) in four different dosages. The colon tissues were collected and RNA extraction and cDNA synthesis were performed. The expression level of fifteen genes related to wound healing was evaluated using the quantitative Real-time PCR.

Fourteen genes were significantly altered in UC models vs. normal controls. Twelve genes were changed after the taper-up-off treatment of opium tincture. The wound healing-related genes were up-regulated after the opium tincture treatment, especially at higher dosages.

The early findings of the gene expression study regarding UC model rats treated with opium tincture suggest that the wound healing pathways activity has been increased after the treatment. It seems that the opium tincture may induce the activation of wound healing with upregulation of related genes including growth factors which may lead to a potential repair of injured tissues such as intestinal mucus that in turn could be a hope for treatment of UC and reduction of the risk of UC induced colon cancer.

Citation: Dezhakam H, Dezhakam A, Dezhakam A, Dezhakam S, Haghighatfard A. Short report of ulcerative colitis rodent model treatment with taper up- off protocol of opium tincture revealed an expression alteration in wound healing genes. Open J Clin Med Images. 2025; 5(2): 1207.

Introduction

Ulcerative Colitis (UC) is a chronic inflammatory bowel disease characterized by inflammation of the colon's mucosa with unclear origins. Epidemiologically, UC exhibits a bimodal age of onset, with peaks typically occurring between 15-25 years and again between 55-65 years of age [1]. The incidence and prevalence of UC vary geographically, with higher rates observed in North America and Europe compared to Asia and Africa, although incidence is rising globally [2]. The etiology of UC is multifactorial and not fully understood, involving a complex interplay of genetic predisposition, environmental factors, and immune dysregulation [3]. Genetic studies have identified numerous susceptibility genes, particularly those involved in immune responses, such as the HLA region [4]. Environmental factors, including alterations in the gut microbiome, and lifestyle factors like diet and smoking, are also implicated in disease development. It is hypothesized that in genetically susceptible individuals, an aberrant immune response to luminal antigens triggers chronic inflammation and damage to the colonic mucosa [5].

Wound healing is a multifaceted process; starts with a series of cellular and molecular procedures and consists of several stages: Hemostasis, inflammation, Proliferation, and remodeling [6]. Growth factors are essential proteins that act as mediators and receptors in the complex process of wound healing, orchestrating the necessary cellular interactions, and are involved in tissue regeneration like fibroblasts, platelets, and epithelial, etc. reduction of growth factors functions may lead to impaired healing process [7].

IL-22, a cytokine, has been identified as playing a role in UC, and animal model studies suggest that IL-22 may protect against colitis by promoting epithelial cell proliferation, mucus production, and antimicrobial peptide production, all of which contribute to intestinal barrier repair and reduced inflammation [8]. IL-22 plays a significant role in this process by promoting the proliferation of epithelial cells, the production of mucus, and the expression of antimicrobial peptides, all of which contribute to the repair of the intestinal barrier [9]. However, IL-22's role is complex and controversial, and chronic overexpression could lead to increased inflammation, colonic neutrophil infiltration, and resistance to ustekinumab therapy [10]. The activity of IL-22 is influenced by various factors including AhR, STAT3, CARD9, Musculin, Treg cells, retinoic acid, and the gut microbiota [11].

In ulcerative colitis, the intestinal mucosal barrier is damaged, and the gut microbiota is dysfunctional. This damage leads to inflammation, with immune cells infiltrating the mucosa and submucosa. Effective wound healing is crucial in UC to repair the damaged tissue and restore normal function [12]. Several factors influence wound healing in UC. STAT3 activation, often triggered by IL-22, is essential for mucosal wound healing. CARD9 also promotes intestinal wound healing, possibly through its regulation of IL-22 [13]. On the other hand, a balanced gut microbiota is important; a decrease in beneficial bacteria and an increase in pathogenic bacteria can impair the healing process [14].

While opium-based medicines are widely used as painkillers, potential side effects, and dependence concerns related to opium, careful considerations regarding these compounds are required. Opium tincture, also known as Laudanum is an opioid-based analgesic medication that also has been used as a treatment for diarrhea, control of neonatal opioid withdrawal syndrome, and used as a cough suppressant [15]. The effects of opium and opiate alkaloids on the gene expression and epigenetic patterns of mammals are not clarified. Gene expression analysis may shed light on the polyhedral of the role of opioid compounds on the whole body including basic molecular pathways such as the immune system, wound healing, and melanogenesis [16].

The present article is the report of early findings of the study aimed to evaluate the transcriptomic pattern of the ulcerative colitis rat model treated with a novel protocol of taper up-off of opium tincture, to understand the potential effects of opium on ulcerative colitis. This early report focuses on the expression level of wound healing-related genes in opium tincture-treated, untreated, and control groups.

Materials and methods

Animal modeling protocol

This study utilized Sprague-Dawley rats, with twelve males and twelve females aged 7-8 weeks in each group. At the start of the study, male body weights ranged from 220 grams to 245 grams, and female body weights ranged from 192 grams to 202 grams. The Dextran Sulfate Sodium (DSS)-Induced acute Colitis used in this study closely mimics the pathology of human UC [17]. Acute colitis was induced by administering 3% DSS in drinking water for a week (Days 0-7). Mice were then given normal drinking water for 5 days before starting the treatment on Day 12. Animals were housed in groups of three per cage with access to ad libitum food. The daily body weights and morbidity were assessed each morning and fecal samples were collected from all rats on sacrifice day. Disease Activity Index (DAI) scores were calculated based on body weight recovery at sacrifice, stool consistency, and the presence of blood in stool. Histopathological damage was scored from H&E-stained distal colon cross-sections, evaluating crypt architecture, inflammatory cell infiltrates, muscle thickening, goblet cell depletion, and crypt abscesses, as previously described [18].

Opium tincture treatment protocol

The rats received opium tincture according to a laboratory-designed taper-up-off protocol, the Dezhakam-Step-Time (DST) method. This method consisted of an 18-step administration schedule, with doses given twice daily for 9 days to taper up, and then twice daily for another 9 days to taper down. Treatment began with the lowest dose, which was then increased by 20% (multiplied by a coefficient of 0.8) at each administration. After 18 administrations (9 days), the process was reversed, with the dose reduced by 20% at each of the following 18 administrations. Consequently, the first and last doses were the same. The experimental groups and their details are outlined in Table 1. Four different dosage regimens were used. As an example, the first regimen started at 2.35 mg/kg, reaching 65.58 mg/kg by the 18th dose. The starting doses for the second, third, and fourth regimens were 3.52 mg/kg, 4.7 mg/kg, and 5.87 mg/kg, respectively.

Scarification and tissue collection

At the end of the experiment, rodents were euthanized by sodium pentobarbital overdose and administered intravenously based on protocols published in previous pharmacology and toxicity studies. Almost a 2-centimeter portion of the distal colon of euthanized rats was collected for molecular analysis. Housing and euthanasia procedures are conducted based on the ARRIVE55 Guidelines checklist and protocols of previous studies [19,20].

Molecular evaluations

The RNA extraction mini kit (Qiagen, Hilden, Germany) was used for total RNA extraction from the rat colon samples according to the manufacturer's instructions. To remove any contaminating genomic DNA, the extracted RNA was treated with DNase I, RNase-free (Fermentas, Latvia; #EN0521) following the manufacturer's protocol. The quantity of the extracted RNA was determined using a Nanodrop-1000 spectrophotometer, and RNA quality was assessed using the BioRad Experion automated electrophoresis system (BioRad Laboratories Inc.). All extracted RNA samples were stored at -80°C until further processing. The cDNA was synthesized using the RevertAid Premium First Strand cDNA Synthesis Kit (Thermo Scientific - Fermentas, Latvia; #K1652), following the manufacturer's protocol.

The candidate genes (Table 2) were quantified using Real-Time PCR. Specific primers and probes were designed using the GenScript Real-time PCR (TaqMan) Primer Design software and their specificity was confirmed by BLAST analysis against the NCBI database. Standard curves were generated using serial four-fold dilutions of pooled cDNA from randomly selected control rat RNA samples. Quantitative Real-Time PCR was performed in triplicate using the CFX96 Touch Real-Time PCR Detection System (BIO-RAD, California, United States). Quality control criteria included the absence of signal in no-template control samples and a standard curve R^2 value greater than 0.99. PCR reaction efficiency was calculated using the Lin-Reg PCR online software (Amsterdam, Netherlands). The TaqMan® PCR Starter Kit (Thermo Scientific - Fermentas, Latvia) was used for all samples. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the housekeeping gene for normalization, and relative gene expression ratios were determined using the Livak ($2^{-\Delta\Delta Ct}$) method.

Statistical assessments

Statistical analyses were conducted using SPSS version 25. The normality of data distribution for all variables was assessed using the Kolmogorov-Smirnov test. One-way ANOVA was employed to determine statistical differences between multiple group comparisons. To control for potential confounding factors, ANCOVA was used to evaluate the persistence of significant differences between groups, with RNA integrity number, cDNA synthesis quality, qPCR plates/runs, and primer and probe efficiency defined as covariates. Bonferroni correction was applied for multiple comparisons. Descriptive data are presented as mean ± Standard Deviation (SD).

Results

Clinical observations

Body weights of all rodents were measured before the first dose administration of DSS-induced colitis and after the UC modeling before the treatment and after the treatment. Results showed that the weight of DSS-induced colitis was significantly lower compared with normal controls (p-value=0.003) which was consequential to DSS treatment and was expected in the colitis animals. During the treatment period, one female rat from G3, and two male and one female rat from the untreated DSS-induced colitis group died before the scarification day.

Gene expression analysis

RNA quality analysis showed all samples had RNA Quality Indicator (RQI) values higher than 9.5. Findings of gene expression comparisons between groups compared with normal control rats have been presented in Table 3. The most significant change in expression level has been detected in G3 and G4. From 15 genes three genes (VEGF165, FGF2, and F3) showed no significant difference between the UC model and treatment groups. All other twelve genes were significantly altered in the G3 and G4 treatment groups compared with the UC model. No significant difference was observed in mRNA levels of any genes between female and male rats in any group.

Table 1: Groups' demographic data.

Group name	Description
G1	DSS-induced colitis model treated with dose 1 opium tincture
G2	DSS-induced colitis model treated with dose 2 opium tincture
G3	DSS-induced colitis model treated with dose 3 opium tincture
G4	DSS-induced colitis model treated with dose 4 opium tincture
ulcerative colitis rodent model (DSS-induced colitis)	DSS-induced colitis model with no treatment
Normal control	Healthy rats with no treatment
Sham	Normal rats with force-feeding of water

Table 2: list of studied genes.

Gene symbol	Full name of gene	Description
TIMP-2	TIMP metallopeptidase inhibitor 2	A metalloproteinase inhibitor
VEGF165	Vascular endothelial growth factor 165	Enhancing the durability within the protease-rich microenvironment of the wound
iNOS	Nitric oxide synthase 2	Undirected activating of multiple members of the VEGF family
Ankrd1	Ankyrin repeat domain 1	Involved in several cellular response related to SMAD receptors
SMAD3	SMAD family member 3	Enables several functions, including DNA-binding transcription factor activity and RNA polymerases
FGF1	Fibroblast growth factor 1	Enables Hsp70 protein binding activity and growth factor activity
FGF-2	Fibroblast growth factor 2	Enables fibroblast growth factor receptor
FGF-5	Fibroblast growth factor 5	Enables growth factor activity
CXCL-1	C-X-C motif chemokine ligand 1	Enables chemokine activity
PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide	Enables phosphatidylinositol 3-kinase binding activity and platelet-derived growth factor alpha-receptor activity
F3	Coagulation factor III	Enables protease binding activity
BMP2	Bone morphogenetic protein 2	Enables growth factor activity
SERPINE1	Serpin family E member 1	Enables protease binding activity and serine-type endopeptidase inhibitor activity
IL-1B	Interleukin-1 beta	Enables cytokine activity
IL-22	Interleukin-22	Predicted to enable cytokine activity

Table 3: Relative mRNA level and statistical comparisons of gene expression evaluations of all groups compared with normal control rats.

Gene	G1	G2	G3	G4	UC model	Sham
TIMP2	Ratio: 0.54 P value:0.002*	Ratio: 0.64 P value:0.003*	Ratio: 0.88 P value:0.09	Ratio: 0.84 P value:0.08	Ratio: 0.43 P value:0.003*	Ratio: 0.96 P value:0.5
VEGF165	Ratio: 0.69 P value:0.004*	Ratio: 0.63 P value:0.004*	Ratio: 0.62 P value:0.007*	Ratio: 0.67 P value:0.003*	Ratio: 0.6 P value:0.002*	Ratio: 0.96 P value:0.71
iNOS	Ratio: 0.71 P value:0.005*	Ratio: 0.68 P value:0.003*	Ratio: 0.62 P value:0.003*	Ratio: 0.69 P value:0.001*	Ratio: 0.48 P value:0.0007*	Ratio: 0.96 P value:0.35
Ankrd-1	Ratio: 0.66 P value:0.004*	Ratio: 0.67 P value:0.004*	Ratio: 0.71 P value:0.005*	Ratio: 0.73 P value:0.007*	Ratio: 0.48 P value:0.0003*	Ratio: 0.9 P value:0.22
SMAD	Ratio:0.68 P value:0.003	Ratio:0.89 P value:0.08	Ratio:0.88 P value:0.2	Ratio: 0.9 P value:0.3	Ratio: 0.5 P value:0.003*	Ratio: 1.2 P value:0.4
FGF2	Ratio:0.66 P value:0.007*	Ratio:0.77 P value:0.007*	Ratio:0.74 P value:0.004*	Ratio: 0.79 P value:0.006*	Ratio: 0.69 P value:0.008*	Ratio: 1.03 P value:0.47
CXCL1	Ratio: 0.82 P value:0.06	Ratio: 0.82 P value:0.06	Ratio: 0.92 P value:0.21	Ratio:1.11 P value:0.3	Ratio: 0.53 P value:0.006*	Ratio: 1.08 P value:0.44
PDGFRA	Ratio: 0.8 P value:0.03	Ratio: 0.9 P value:0.06	Ratio: 1.2 P value:0.09	Ratio:1.16 P value:0.09	Ratio:0.57 P value:0.007*	Ratio: 1.02 P value:0.7
FGF-5	Ratio: 0.66 P value:0.005*	Ratio: 0.9 P value:0.05	Ratio: 0.96 P value:0.06	Ratio: 1.2 P value:0.3	Ratio: 0.5 P value:0.009*	Ratio: 1.1 P value:0.36
F3	Ratio: 0.88 P value:0.17	Ratio: 0.89 P value:0.23	Ratio: 0.95 P value:0.14	Ratio: 0.91 P value:0.3	Ratio: 1.06 P value:0.4	Ratio: 0.97 P value:0.47
BMP2	Ratio: 0.65 P value:0.007*	Ratio: 0.68 P value:0.005*	Ratio: 0.92 P value:0.11	Ratio: 1.2 P value:0.2	Ratio: 0.54 P value:0.002*	Ratio: 1.06 P value:0.75
SERPINE1	Ratio: 1.33 P value:0.006*	Ratio: 1.2 P value:0.007*	Ratio: 0.97 P value:0.09	Ratio: 0.91 P value:0.1	Ratio: 1.48 P value:0.001*	Ratio: 0.96 P value:0.2
FGF1	Ratio: 0.66 P value:0.003*	Ratio: 0.89 P value:0.05	Ratio: 1.1 P value:0.06	Ratio: 1.2 P value:0.05	Ratio: 0.49 P value:0.003*	Ratio: 0.93 P value:0.08
IL1B	Ratio: 1.08 P value:0.06	Ratio: 1.05 P value:0.05	Ratio: 1.01 P value:0.11	Ratio: 0.94 P value:0.13	Ratio: 1.59 P value:0.001*	Ratio: 0.99 P value:0.5
IL22	Ratio: 1.28 P value:0.005*	Ratio: 1.07 P value:0.09	Ratio: 0.91 P value:0.2	Ratio: 0.96 P value:0.2	Ratio: 1.45 P value:0.004*	Ratio: 0.89 P value:0.08

*refer to statistical significance (after multiple comparison correction testing)

Discussion

Results of all gene expression analyses showed that most of the wound healing-related genes were down-expressed in UC models and activated or upregulated during the treatment of all four groups while their activation was mostly dose-dependent. On the other hand, genes related to the immune system including IL22, IL1b, and SERPINE1 were highly over-expressed in UC models and were downregulated during the treatments in turn may reduce the UC-induced inflammation and help the wound healing process activations [21].

SERPINE1, IL1b, and IL22 are involved in response to several immune system and inflammation processes. SERPINE1 is involved in cellular response to cytokine stimulus and injury-induced inflammation pathways. The compromised intestinal mucosal barrier in ulcerative colitis initiates an intricate molecular cascade crucial for tissue repair. A central mediator in this healing process is the cytokine Interleukin-22 (IL-22), which stimulates epithelial cell proliferation, mucus production, and antimicrobial peptide expression, collectively aiding in the restoration of the intestinal barrier. IL-22 primarily functions via STAT3 activation, a transcription factor that governs the expression of numerous wound healing-related genes. Additionally, other molecules, such as CARD9, also participate in intestinal wound healing, potentially by influencing IL-22 production [22].

TIMP2 is a metalloproteinase (MMP) inhibitor, involved in several processes, including positive regulation of adenylate cyclase activity and regulation of intracellular signal transduction [23]. The iNOS enables heat shock protein 90 binding activity and enzyme binding activity and is involved in activating multiple members of the VEGF family and improving revascularization and the rate of wound healing [24]. Ankrd1 has essential effects on wound neovascularization and endothelial migration in several animal models and enables R-SMAD binding activity; cytoskeletal protein binding activity; and transcription co-regulator activity. The Ankrd1 and SMADs are involved in DNA-binding transcription factor activity; RNA polymerase II transcription regulatory region sequence-specific DNA binding activity; and beta-catenin binding activity [25]. The Beta-catenin's binding activity in turn plays a major role in cell adhesion and signaling, through the Wnt pathway [26] and regulation of macromolecule metabolic process regulating inflammation in chondrocyte differentiation and cutaneous tissue repair [27]. FGF1 and FGF5 are active in the cytoplasm and extracellular space, and act upstream of signal transduction, activation of fibroblast growth factor receptor signaling pathway; positive regulation of MAPK cascade; and positive regulation of cell population proliferation [28]. CXCL1, located in extracellular space is involved in positive regulation of monoatomic ion transport [29]. The PDGFRA protein is located in several cellular components, including axon; nucleus; and plasma membrane [30]. BMP2 is involved in the BMP signaling pathway; and cellular response to mechanical stimulus and positive regulation of transcription by RNA polymerase II [31].

Previous studies showed that the Taper up-off method (DST method) may reduce the risk of opium dependence disorder and may suggest a potentially safe way to benefit from medical applications of opioid-based compounds of opium tincture [32,33]. While the effects of opium tincture taper up-off on UC and UC-induced molecular pathways including inflammation and wound healing are not clarified our early results may suggest that using the new protocol of opium tincture oral consumption could have reduced the inflammation of colon tissue on UC models along with activation of wound healing processes, that in turn may lead to improvement of disease and prevention from colon cancer.

References

1. O'Shea MD, Kaplan GG. Epidemiology of inflammatory bowel disease. Gastroenterol Clin North Am. 2016; 45: 147–58.
2. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012; 142: 46–54.e42.
3. Ungaro R, Bernstein CN, Gearry RB, Hobert AM, Ng SC, Annese V, et al. Ulcerative colitis: pathophysiology, diagnosis, and management. Nat Rev Gastroenterol Hepatol. 2017; 14: 690–707.
4. Andersen V, Tyazhelov E, Jørgensen T. Genetics and epigenetics of inflammatory bowel disease. World J Gastroenterol. 2011; 17: 187.
5. Preda CM, Istrătescu D. Etiology of Ulcerative Colitis. In: Ulcerative Colitis-Etiology, Diagnosis, Diet, Special Populations, and the Role of Interventional Endoscopy. IntechOpen; 2022 Sep 1.
6. Nurkesh A, Jaguparov A, Jimi S, Saparov A. Recent advances in the controlled release of growth factors and cytokines for improving cutaneous wound healing. Front Cell Dev Biol. 2020; 8: 638.
7. Miricescu D, Badoiu SC, Stanescu-Spinu II, Totan AR, Stefani C, Greabu M. Growth factors, reactive oxygen species, and metformin—promoters of the wound healing process in burns?. Int J Mol Sci. 2021; 22: 9512.
8. Zhao N, Liu C, Li N, Zhou S, Guo Y, Yang S, et al. Role of Interleukin-22 in ulcerative colitis. Biomed Pharmacother. 2023; 159: 114273.
9. Arshad T, Mansur F, Palek R, Manzoor S, Liska V. A double edged sword role of interleukin-22 in wound healing and tissue regeneration. Front Immunol. 2020; 11: 2148.
10. Pavlidis P, Tsakmaki A, Pantazi E, Li K, Cozzetto D, Digby-Bell J, et al. Interleukin-22 regulates neutrophil recruitment in ulcerative colitis and is associated with resistance to ustekinumab therapy. Nat Commun. 2022; 13: 5820.
11. Bai J, Bai J, Yang M. Interleukin-22 attenuates acute pancreatitis-associated intestinal mucosa injury in mice via STAT3 activation. Gut Liver. 2021; 15: 771.
12. Çavdar M, Çavdar M. Alternative therapeutic applications used in the treatment of ulcerative colitis: probiotics, prebiotics, synbiotics and fecal microbiota transplantation. Prog Nutr. 2023; 25.
13. Danne C, Lamas B, Lavelle A, Michel ML, Da Costa G, Pham HP, et al. Dissecting the respective roles of microbiota and host genetics in the susceptibility of Card9−/− mice to colitis. Microbiome. 2024; 12: 76.
14. Liu T, Guo Y, Liao Y, Liu J. Mechanism-guided fine-tuned microbiome potentiates anti-tumor immunity in HCC. Front Immunol. 2023; 14: 1333864.
15. Okdahl T, Høyer KL, Knoph CS, Davidsen L, Larsen IM, Mark EB, et al. Opium tincture has anti-propulsive effects in patients with chronic diarrhea: a randomized, placebo-controlled, and crossover trial. Scand J Gastroenterol. 2024; 59: 1023–34.
16. Dezhakam H, Dezhakam A, Dezhakam A, Dezhakam S, Haghighatfard A. Short report of potential Myelinogenesis effects of taper up-off of opium tincture in rodent model of multiple sclerosis. J Neurol Neurol Sci Disord. 2024; 10: 021–6.

17. Kiesler P, Fuss IJ, Strober W. Experimental models of inflammatory bowel diseases. Cell Mol Gastroenterol Hepatol. 2015; 1: 154–70.

18. Tusé D, Reeves M, Royal J, Hamorsky KT, Ng H, Arolfo M, et al. Pharmacokinetics and safety studies in rodent models support development of EPICERTIN as a novel topical wound-healing biologic for ulcerative colitis. J Pharmacol Exp Ther. 2022; 380: 162–70.

19. Yang T, Ma X, Wang R, Liu H, Wei S, Jing M, et al. Berberine inhibits IFN-γ signaling pathway in DSS-induced ulcerative colitis. Saudi Pharm J. 2022; 30: 764–78.

20. Shomer NH, Allen-Worthington KH, Hickman DL, Jonnalagadda M, Newsome JT, Slate AR, et al. Review of rodent euthanasia methods. J Am Assoc Lab Anim Sci. 2020; 59: 242–53.

21. Zhu F, Yang T, Ning M, Liu Y, Xia W, Fu Y, et al. MiR-146a alleviates inflammatory bowel disease in mice through systematic regulation of multiple genetic networks. Front Immunol. 2024; 15: 1366319.

22. Martín Adrados B. Intestinal epithelial cell response to Endoplasmic Reticulum stress in Inflammatory Bowel Disease.

23. Jian F, Yanhong J, Limeng W, Guoping N, Yiqing T, Hao L, et al. TIMP2 is associated with prognosis and immune infiltrates of gastric and colon cancer. Int Immunopharmacol. 2022; 110: 109008.

24. Sakthivel KM, Guruvayoorappan C. Amentoflavone inhibits iNOS, COX-2 expression and modulates cytokine profile, NF-κB signal transduction pathways in rats with ulcerative colitis. Int Immunopharmacol. 2013; 17: 907–16.

25. Ou W, Xu W, Liu F, Guo Y, Huang Z, Feng T, et al. Increased expression of yes-associated protein/YAP and transcriptional coactivator with PDZ-binding motif/TAZ activates intestinal fibroblasts to promote intestinal obstruction in Crohn's disease. EBioMedicine. 2021; 69.

26. Liu J, Xiao Q, Xiao J, Niu C, Li Y, Zhang X, et al. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther. 2022; 7: 3.

27. Zhang C, Liu LW, Sun WJ, Qin SH, Qin LZ, Wang X. Expressions of E-cadherin, p120ctn, β-catenin and NF-κB in ulcerative colitis. J Huazhong Univ Sci Technol Med Sci. 2015; 35: 368–73.

28. Katoh M, Katoh M. FGF signaling network in the gastrointestinal tract. Int J Oncol. 2006; 29: 163–8.

29. Zhang RB, Dong LC, Shen Y, Li HY, Huang Q, Yu SG, et al. Electroacupuncture alleviates ulcerative colitis by targeting CXCL1: evidence from the transcriptome and validation. Front Immunol. 2023; 14: 1187574.

30. Kim TW, Hong HK, Lee C, Kim S, Lee WY, Yun SH, et al. The role of PDGFRA as a therapeutic target in young colorectal cancer patients. J Transl Med. 2021; 19: 1–3.

31. Karagiannis GS, Afaloniati H, Karamanavi E, Poutahidis T, Angelopoulou K. BMP pathway suppression is an early event in inflammation-driven colon neoplasmatogenesis of uPA-deficient mice. Tumour Biol. 2016; 37: 2243–55.

32. Dezhakam H, Dezhakam A, Dezhakam S, Dezhakam A, Haghighatfard A. A new protocol of methamphetamine dependence treatment with taper-up-off treatment of opium tincture, a new hope to cure the methamphetamine addiction. Int J Psychiatry. 2023; 8: 88–92.

33. Dezhakam H, Dezhakam A, Dezhakam S, Haghighatfard A. A new taper off treatment of opium dependents can lead to cure the addiction as well as improvement of cognitive functions.