國立臺灣海洋大學食品科學系
碩士班專題討論書面報告

探討不同藻類多醣減緩小鼠結腸炎之作用

To investigate the effects of different algaeale polysacharides on attenuating colitis in mice

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Outline

1. **Introductioon**
2. **Dietary polysaccharide-rich extract from *Eucheuma cottonii* modulates the inflammatory response and suppresses colonic injury on dextran sulfate sodium-induced colitis in mice**
3. **Potent anti-inflammatory activity of polysaccharides extracted from *Blidingia minima* and their effect in a mouse model of inflammatory bowel disease**
4. **Conclussions**

Abstract

Inflammatory bowel disease (IBD) is a known medical burden in most developed countriesand and a significant cause of morbidity. Now pharmacological and surgical intervention are the two main management approaches for IBD. However, some drugs result in drug resistance and side effects. Therefore, natural products, including those with marine origins have been investigated for the improvement of IBD clinical symptoms. In the first study, colitis was induced in mice by the administration of dextran sulfate sodium (DSS), and then DSS-induced mice were treated with *Eucheuma cottonii* (EC) extracts or curcumin as a positive control. Mice were sacrificed post-treatment and blood samples were collected. The disease activity index (DAI) and inflammatory cytokine levels were measured. After treatment, EC extract administration protected against weight loss and decreased the colon weight perlength ratio, it also decreased pro-inflammatory cytokine expression, increased IL-10 levels, and reduced colonic damage. In the second study, in vitro and in vivo experiments were performed using dextran sulfate sodium (DSS)-treated intestinal epithelial cells and mice with DSS-induced colitis, respectively. The mice in both treatment groups were treated by oral gavage with either saline or *Blidingia minima* polysaccharides (BMP) once a day for 7 days. At the end of the experiment, colon lengths and weights were recorded, blood and colonic samples were collected for further analysis. BMP showed an anti-inflammatory effect on DSS-treated cells. Furthermore, BMP alleviated colitis symptoms in DSS-treated mice, it also repaired colonic dysfunction, and colonic morphology, infiltration, and the expression of tight junction proteins were all improved. Therefore, both of EC extracts and BMP can reduce DSS-induced bowel inflammation, thereby becoming a promising candidate for the treatment of colitis.

一、前言

發炎性腸道疾病 (Inflammatory bowel disease, IBD) 是一種包括克羅恩病 (Crohn's disease, CD) 和潰瘍性結腸炎 (ulcerative colitis, UC) 的總稱，在大多數已開發國家是一個已知的醫療負擔。潰瘍性結腸炎的特點是腸道發炎，通常會導致腹瀉、血性黏稠、體重減輕和結腸縮短 (Hendrickson ,*et al.*, 2002; Cosnes *et al.*, 2011; Bitencourt *et al.*, 2015)。自 20 世紀中葉以來，IBD 的發病率在西方世界 (例如北美、歐洲、新西蘭和澳大利亞) 有所增加，直到最近才在亞洲、中東和南美新興工業化國家發現 IBD 的病患。此外，在印度和中國等人口眾多、快速城市化和西方化的新興工業化國家，IBD 患病率也在增加。因此，IBD 可被歸類為一種全球性疾病 (Molodecky *et al.*, 2012; Kaplan *et al.*, 2015)。

目前發炎性腸道疾病有藥物和外科手術兩種主要治療方法 (Lean *et al.*, 2015)。硫唑嘌呤 (azathioprine)、6-巰嘌呤 (6-mercaptopurine) 和抗生素等傳統治療藥物在類固醇抗性和類固醇依賴患者中變得越來越重要 (Cho *et al.*, 2011)。皮質類固醇、胺基水楊酸和免疫抑製劑等藥物旨在減少炎症，但對長期緩解的療效有限，並且存在明顯的副作用 (Lean *et al.*, 2011; Triantafillidis *et al.*, 2011; Lean *et al.*, 2015)。因此，源自海洋的天然產品，成為改善 IBD 臨床症狀的潛在候選者。

一些海藻萃取物已被證實具治療 IBD 的潛力，包括墨西哥蕨藻多醣，一種來自鍵狀茨藻的硫酸化多醣，以及來自墨角藻的岩藻聚醣提取物 (Bitencourt *et al.*, 2015; Lean *et al.*, 2015; Brito *et al.*, 2016)。許多海藻富含生物活性化合物，如多醣、萜烯和類黃酮，已被證明具有抗腫瘤、抗原蟲、抗病毒、抗氧化、抗傷害、抗炎和抗凝血等藥理活性 (De Souza E´ T *et al.*, 2009; da Matta *et al.*, 2011; Torres *et al.*, 2014; Bitencourt *et al.*, 2015)。海藻中含有大量的多醣，但由於胃腸道中缺乏所需的酶，大部分不能被人體消化，因此可以被視為膳食纖維，佔海藻組成含量約 33-75% (Jime´nez-Escrig *et al.*, 2000; Go´mez-Ordo´ñez *et al.*, 2010)。紅藻多醣中主要由鹿角菜膠和瓊脂等硫酸化半乳聚醣組成，且被用於評估治療結腸炎和調節腸道微生物群之潛力(Rodrı´guez-Cabezas *et al.*, 2001; Galvez *et al.*, 2005; Koh *et al.*, 2016; Makki *et al.*, 2018)

在嚙齒動物模型中可用多種化學試劑誘發結腸炎，包括硫酸鈉聚葡萄醣 (dextran sodium sulfate, DSS)、三硝基苯磺酸 (trinitrobenzene sulfonic acid, TNBS)、唑啉酮 (oxazolone)、乙酸 (acetic acid)、吲哚美辛 (indomethacin)、和肽聚醣多醣 (Randhawa *et al.*, 2014)。透過加入 DSS 的飲用水可誘發急性或慢性結腸炎，並具劑量和時間相關性 (Park *et al.*, 2015)。而 DSS 誘導的結腸炎模型因其可控性、可重複性、簡單性和快速性而廣被使用 (Chassaing *et al.*, 2015)，並且在生化和組織形態學上都已被證實可代表人類結腸炎 (Jeon *et al.*, 2016)。

二、富含膳食多醣的耳突麒麟菜萃取物調節發炎反應與抑制聚葡萄糖硫酸鈉誘導小鼠結腸炎的結腸損傷

耳突麒麟菜 (*Eucheuma cottoni*, EC) 是一種紅藻，也被稱為海鳥巢，先前曾有報導稱其具有抗氧化、抗凝血、抗腫瘤和抗炎特性 (Kumar *et al.*, 2008; Matanjun

et al., 2008; Amvar *et al*., 2012; ng *et al*., 2014; Abu-Bakar *et al*., 2015)。此外，從這種海藻中提取的提取物可減緩腫瘤細胞的生長速度 (Chang *et al*., 2017)，促進傷口癒合 (Fard *et al*., 2011) ，並促進癌細胞凋亡 (Abu-Bakar *et al*., 2017)。EC 萃取物也已被證明可以改善肥胖大鼠模型的心血管、肝臟和代謝相關生化參數 (Wanyonyi *et al*., 2017)，並在鏈佐黴素誘導的 2 型糖尿病小鼠中表現出抗糖尿病作用 (Lee *et al*., 2017) 。因此，本文旨在研究從 EC 中提取富含多醣的萃取物對結腸炎模型的影響。

將耳突麒麟菜乾燥粉末於 50°C 下以乙醇浸漬萃取 3 小時，再經過濾將萃取物與殘留物分離，總共進行三次提取，將乙醇萃取物揮發乙醇後使用冷凍乾燥機凍乾，獲得 EC 萃取物。以凱氏定氮分析 EC 萃取物總氮含量，並分析測定水分含量、粗蛋白百分比、粗脂肪含量及灰份含量分別利用碳水化合物含量，結果得碳水化合物 (74.77 ± 1.15%) 和灰分 (19.66 ± 0.16%)，其次是蛋白質 (2.88 ± 0.05%)、水分 (1.44 ± 0.48%) 和脂肪 (1.26 ± 0.18%)，其結果可知碳水化合物占比最多。

為了解不同濃度 EC 萃取物對細胞活性的影響，使用 RAW 264.7 巨噬細胞與不同濃度 (125、250、500、1000 μg/mL) 的 EC 萃取物及沒有添加萃取物的控制組在 96 孔微孔板中培養 24 小時，進行 MTT 測試。結果得 EC 提取物對 RAW 264.7 巨噬細胞 (Fig. 1) 沒有毒性，且 EC 萃取物和對照處理的細胞之間的細胞活性沒有顯著差異。此海藻萃取物於先前報導顯示無顯著細胞毒性 (Namvar *et al*., 2013; Lim *et al*., 2015)

於動物實驗設計的部分，先將 48 隻六週齡雄性 BALB/c 小鼠，以每籠 4 隻餵養適應一周，之後隨意分為 6 組，每組 8 隻，其中一組未接受 DSS (控制組)，另外 5 組在飲水中給予 2.5% (w/v) DSS 持續 7 天，7 天後每日經口投予不同劑量的 EC 萃取物 (DSS+EC：10.35 g/kg b.w.、DSS+EC2：0.70 g/kg b.w.、DSS+EC5：1.75 g/kg b.w.)、薑黃素 (DSS+Cur ：0.10 g /kg b.w.)，或水 (DSS) (Fig. 2) 每日口服一次，持續 7 天，並每日檢查小鼠體重、糞便稠度和血便的狀況。在第 8 天犧牲前禁食 12 小時，當天測量結腸重量和長度，並將血清和結腸組織儲存在-20°C。作為陽性對照組的薑黃素已被證明可通過抑制 NF-κB (Brumatti *et al*., 2014) 和抑制 STAT3 信號傳導 (Yang *et al*., 2013) 來預防結腸炎。此外，薑黃素還可以調節某些發炎介質，例如腫瘤細胞壞死因子 (Tumor necrosis factor, TNF-α) 和一氧化氮 (Arafa *et al*., 2009)。

因 DSS 所引起的結腸炎有體重減輕、腹瀉和直腸出血等急性組織學變化等，因此測量小鼠體重及其疾病活動指數，患有 DSS 誘導結腸炎的小鼠體重明顯下降，而 EC 萃取物與薑黃素皆有助於減緩體重下降 (Fig. 3a)。此外，結腸炎小鼠的疾病活動指數透過計算 DAI 值，以 [(體重減輕評分) + (大便稠度) + (直腸出血評分)]/ 4 做為計算，得知未經治療的小鼠疾病活動指數評分相較高於 EC 和薑黃素治療組 (Fig. 3b)，且兩項結果都與先前研究一致 (Kim *et al*., 2013; Chassaing *et al*., 2015; Jeon *et al*., 2016)。

結腸炎的特徵還有結腸縮短 (Fig. 4a、4c) 和增加的結腸重量/長度比 (Fig. 4b)。經 DSS 處理組，在 7 天後，小鼠的結腸長度減少，而給予高劑量的 EC 萃取物或薑黃素組別顯著降低結腸縮短。此外，這兩種治療還導致結腸重量/長度比降低 (Fig. 4b) 和脾臟重量降低 (Fig. 4c)。總體而言，用 EC 萃取物或薑黃素治療改善了 DSS 在小鼠中誘導的結腸炎的臨床症狀和病徵。

以 ELISA 分析血清中細胞激素含量，促發炎相關細胞激素 interleukin-1β (IL-1β)、interleukin-6 (IL-6)及 TNF-α，結果顯示，DSS 組與控制組相比這些激素濃度顯著增加 (Fig. 5a, b, c)。用 EC 萃取物或薑黃素處理可降低血清中的 TNF-α、IL-1β 和 IL-6 濃度。此外，EC 提取物還顯著降低了結腸組織中 IL-1β 的表現 (Fig. 6a)，DSS 組與控制組相比，其結腸組織中抗發炎細胞激素 IL-10 顯著下降 (Fig. 6b)。

觀察小鼠腸道組織病理學，於 H&E 染色之結果可看出正常結腸顯示出良好的壁層結構，並且沒有腺窩細胞減少。然而，DSS 組的小鼠表現出較厚的黏膜層並伴有糜爛 (Fig. 7)。用高劑量 EC 萃取物或薑黃素治療後，腺窩細胞減少得到改善。

綜述以上，EC 萃取物有減緩小鼠體重下降、腸道組織中的壁層結構損傷及結腸縮短等作用，且血清中促發炎細胞激素 TNF-α、IL-1β 和 IL-6 也有下降的趨勢。

三、從盤苔中萃取有效抗發炎活性多醣及其在發炎性腸道疾病小鼠模型中之作用

盤苔 (*Blidingia minima*) 在許多海岸線上普遍存在，因這種綠藻生長於海水鹽度和淡水潮汐區變化很大的地區，常與石蓴 (*Enteromorpha*) 一起生長，但盤苔的細胞直徑更小。近年，石蓴的萃取物，特別是多醣類已經被證實具有許多有益的影響，例如抗發炎、抗氧化和降血脂活性。(Cho, Lee *et al*., 1997; Teng *et al*., 2013)。紫菜的繁殖也加劇了盤苔的傳播，因紫菜可為附著的基質，所以盤苔常被認為是海岸的污染物，因其對紫菜產品的質量造成危害。但將盤苔當作廢棄物進行處理不僅昂貴也需大量的人力。

從蘇北淺灘 (中國江蘇省) 的水產養殖中刮取附著的盤苔 (Fan *et al*., 2015)。在現場將樣品初步清理曬乾過後，再進一步用流水沖洗並在 60 ℃ 下乾燥研磨，接著以 90^{o}C 連續攪拌下用蒸餾水萃取用 1kg 盤苔粉末 3 小時，而後冷卻至 20℃。經離心及矽藻土過濾，最後收集上清液並送入吸附層析管柱，用蒸餾水以 60 mL/min 的流速洗滌提取物，而後將溶析液在燒瓶中用旋轉蒸發儀乾燥。最後將濃縮的上清液與 5 L 乙醇混合 24 小時，然後以 8000×g 離心 10 分鐘。去除上清液，冷凍乾燥後獲得萃取物 (polysaccharides extracted from *Blidingia minima*，BMP)。

將 BMP 進行分析，可知 BMP 的總糖、總蛋白、醣醛酸和硫酸鹽含量分別為 491.4、196.1、100.2 和 137.7 mg/g，而滸苔 (*teromorpha prolifera*, EPP) 多醣的總糖、蛋白質、醣醛酸和硫酸鹽含量分別為 546、101、124 和 179 mg/g (Teng *et al*., 2013)，可發現數據非常相似。此外，之前的研究聲稱 EPP 的主要成分由鼠

李糖、木糖、甘露糖、半乳糖胺和葡萄糖組成，而 BMP 主要由鼠李糖、木糖、葡萄醣醛酸和葡萄糖組成，還有少量的甘露糖，半乳糖胺和半乳糖。 因此，EPP 和 BMP 具有相似的組成。

為了測定 BMP 對細胞活性之影響，作者將豬的小腸上皮 (IPEC-J2) 細胞與不同濃度的 BMP (分別為 10、20 和 40 μg/mL) 和 DSS (20 μg/mL) 處理 24 小時，並進行 MTT 試驗及收集細胞之 RNA 以分析促發炎細胞激素表達。結果發現，BMP 處理 IPEC-J2 細胞後，細胞活性沒有顯著變化 (Fig. 8A)，代表 BMP 不會影響細胞活性，而在 DSS 組的促發炎細胞激素 IL-1β 和 TNF-α mRNA 表現相較於控制組有顯著性的增加，BMP 組與 DSS 組相比，在抗發炎細胞激素 IL-10 的表現有顯著性的增加，顯示出 BMP 潛在的抗炎作用。

於動物實驗中，將 32 隻 10 週齡的 C57BL/6J 雄性小鼠，保持在 22 ± 2°C，相對濕度為 50 ± 5% 的環境中。所有的小鼠都任意進食，自由飲水，並保持在 12 小時光照/12 小時黑暗循環下。隨機分為兩組，分別為新鮮自來水 (控制組) 及含 3.5%DSS 的水 (實驗組)。兩組中的小鼠每日進行一次管餵生理食鹽水或不同濃度 (分別為 10、20 和 40 μg/mL) 之 BMP (2 mg/kg b.w.)，持續 7 天。實驗結束時，採取眼窩靜脈竇血液，並經 8000 g，4°C 離心 10 min，獲得血清。此後進行頸脫臼安樂死，記錄結腸長度和重量，並收集結腸樣品用於進一步分析。

基於體重變化、直腸出血、糞便稠度及潛血和動物整體狀況計算疾病活動指數 (DAI)。體重損失評分為：0，無體重減輕；1，體重較減輕 1-5%；2，體重減輕 5–10%；3，體重減輕 10–20%；4，體重減輕 >20%。對於糞便稠度，形狀良好的顆粒得分為 0 分，不粘附在肛門上的糊狀和半成型糞便得分為 2 分，粘附在肛門上的液體糞便得分為 4 分。對於 OB，無血得分為 0，OB 陽性為 2 分，大出血為 4 分。將這些分數相加並除以 3，得出 DAI 值 (Han *et al.*, 2015)。 經 BMP 治療的組別與 DSS 組相比，評分有顯著性的下降 (Fig. 9C)，代表 BMP 有助於減緩結腸炎的症狀。

為探討 BMP 對於結腸組織病理變化中的作用情況，以 HE 染色和穿透式電子顯微鏡進行分析。並使用以下標準對結腸炎的組織學等級進行評分：損傷 (無，0；線窩基底 1/3 缺失，1；線窩基底 2/3 缺失，2；整個腺窩缺失但上皮細胞表面完整，3；整個腺窩和表面上皮細胞的損失，4)。病變範圍大小 (無，0；局灶性，1；涉及 1/3 腸道的病變，2；涉及 2/3 腸道的病變，3；涉及整個腸道的病變，4)。DSS 誘導組的小鼠結腸絨毛表現出部分丟失和脫落 (Fig. 10)，而投予 BMP 的小鼠結腸絨毛沒有此病理的變化。此外，與其他三組小鼠相比，DSS 組別中，其結腸炎組織學指數有顯著升高 (Fig. 10)，代表經 DSS 誘導後小鼠經投予 BMP 可以減緩結腸組織的發炎反應.。

為了觀察腸道通透性，在小鼠犧牲前 4 小時，經口投予 FITC-dextran，並於激發和發射波長分別為 488 和 525 nm 下偵測血清中螢光表現 (Zhang *et al.*, 2015)，因 FITC-dextran 不會被腸道消化吸收，因此若腸道出現滲漏，即可在血液中檢驗出。DSS 組小鼠血清中的 FITC 螢光強度顯著高於對照小鼠和 DSS-BMP

組的小鼠 (Fig. 11A)。此外，DSS 處理組導致二胺氧化酶 (Diamine oxidase, DAO) 活性降低，組織胺無法被代謝，進而造成腸道發炎。血清中內皮素 1 (endothelin 1, ET-1) 濃度升高，腸道可能有出血症狀，導致內皮素增加增進血管收縮。結腸中骨隨氧化酶 (myeloperoxidase, MPO) 是發炎反應時，主要催化產生組織氧化損傷物質的酵素，嗜酸性白血球之過氧化酶 (eosinophil peroxidase, EPO) 也被證明能催化亞硝酸鹽的氧化，產生劇毒活性氮物質，而 DSS 誘導組 MPO 和 EPO 表現皆顯著升高 (Fig. 11B-E)，以 BMP 治療的小鼠中沒有觀察到這種變化。由此可知 BMP 可以減少經 DSS 誘導的發炎反應及有改善腸道屏障的功能。

為了解腸道黏膜細胞之狀況，檢測緊密連接蛋白 (tight junction protein) 的表現，包括 ZO-1、occludin 和 claudin-1。DSS 處理組與和對照組的小鼠相比，小鼠結腸中 ZO-1、occludin 和 claudin-1 的蛋白表現有顯著性的降低 (Fig. 12)，而投予 BMP 治療可減輕 DSS 處理小鼠中 ZO-1、occludin 和 claudin-1 蛋白表現的降低，可知 BMP 緩解 DSS 誘導抑制緊密連接蛋白的表現。

收集小鼠血清並以 ELISA 檢測其促炎細胞激素 TNF-α 及 IL-1β以及抗炎細胞激素 IL-10 的濃度。DSS 組與其他組相比，促炎細胞激素 TNF-α 及 IL-1β 顯著性的上升，而 IL-10 則有顯著性的下降 (Fig. 13)。同時，從結腸組織中提取總 RNA 並以 qRT-PCR 分析上述細胞激素，與上述趨勢相同。

使用西方墨點法測驗結腸中 NF-κB 路徑的活化，如 Fig. 14 所示，與對照組和 DSS-BMP 組相比，DSS 組的小鼠結腸中 NF-κB、IκB-α 和磷酸化 AKT 的蛋白質表現顯著增加。與對照小鼠相比，投予 BMP 7 天後，DSS 誘導的 NF-κB、IκB-α 和 AKT 的蛋白質表現沒有顯著差異，代表 BMP 能減緩 NF-κB 分泌促發炎相關細胞激素，減少發炎反應。

綜述以上，經 DSS 誘導小鼠結腸炎後投予 BMP，能減緩發炎反應，並可以減輕腸道黏膜緊密連接蛋白的減少，降低腸道腺窩及絨毛的損傷，維持腸道屏障的完整度。

四、結論

盤苔及耳突麒麟菜之萃取多醣對細胞皆不會造成毒性，且能減緩結腸炎小鼠體重的減輕、降低疾病活性指數維持腸道屏障，並減緩腸道的發炎反應，表明兩者皆具做為治療 IBD 之應用潛力。

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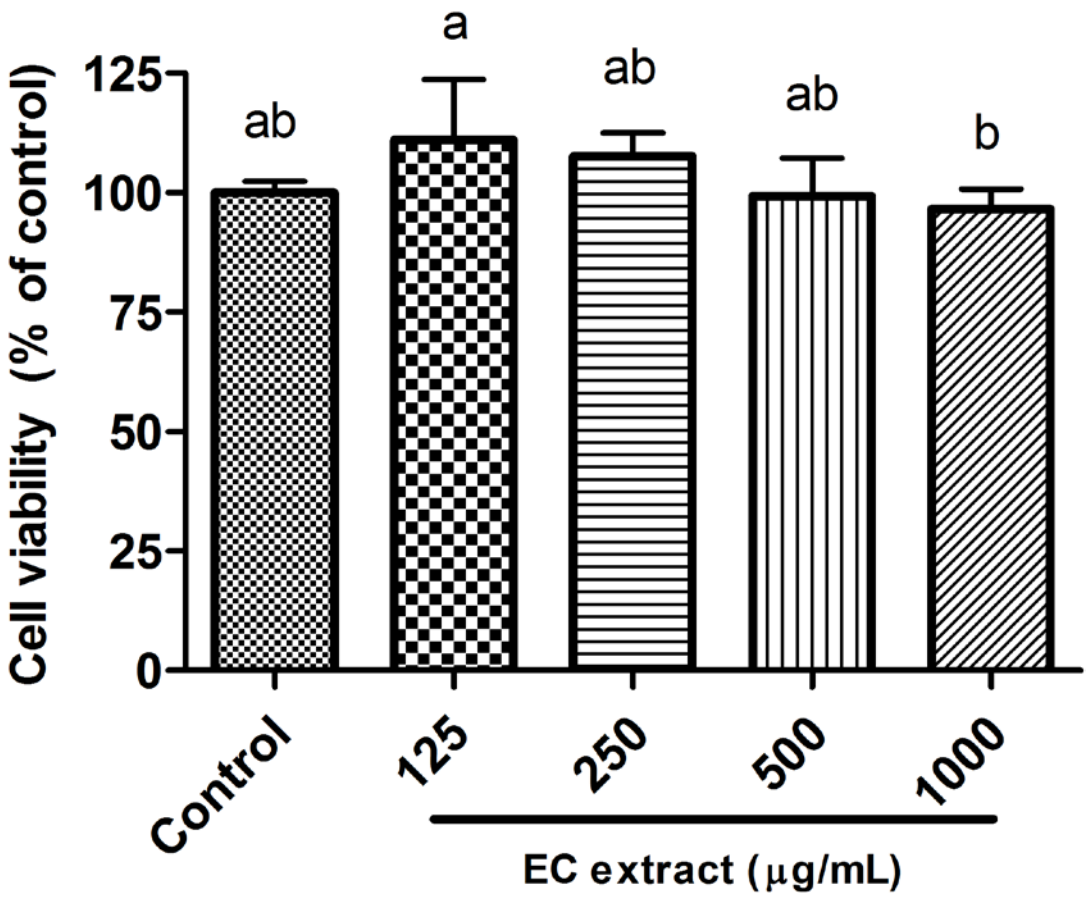


Fig 1. Cell viability of RAW 264.7 macrophage after treatment with EC extract for 24 h. Data are shown as the mean ± S.D. (n = 5). Different letters (a-b) indicate statistical significance (P<0.05) as determined by Duncan's multiple range test. (Sudirman *et al*., 2018)

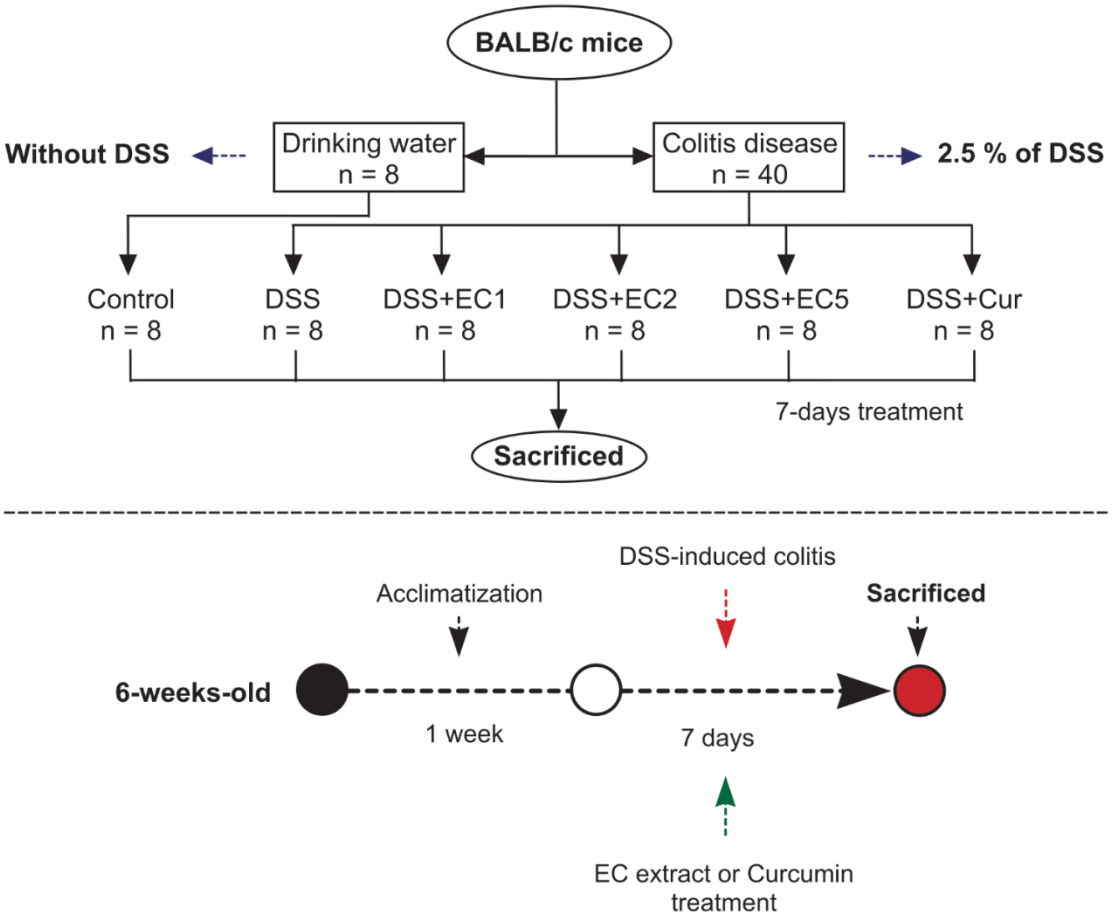
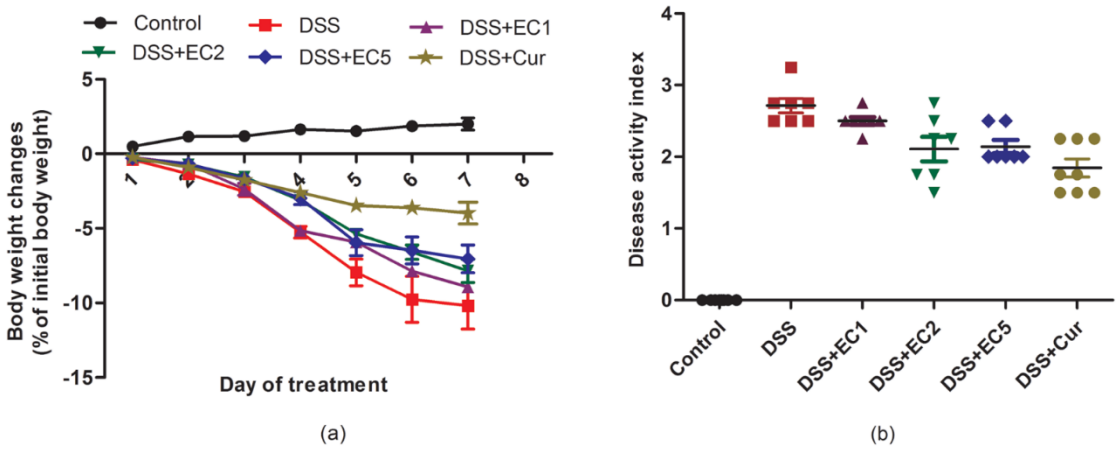


Fig 2. Flowchart of dextran sulfate sodium (DSS) induction of colitis in mice.

(Sudirman *et al*., 2018)



(a) (b)

Fig 3. Effect of EC extract on (a) weight loss and (b) disease activity index in DSS-treated mice during and after 7 days of treatment. Data are shown as the mean ± S.D. (n = 8). Letters indicate statistical significance (P<0.05) as analyzed by Duncan's multiple range test.

(Sudirman *et al.*, 2018)

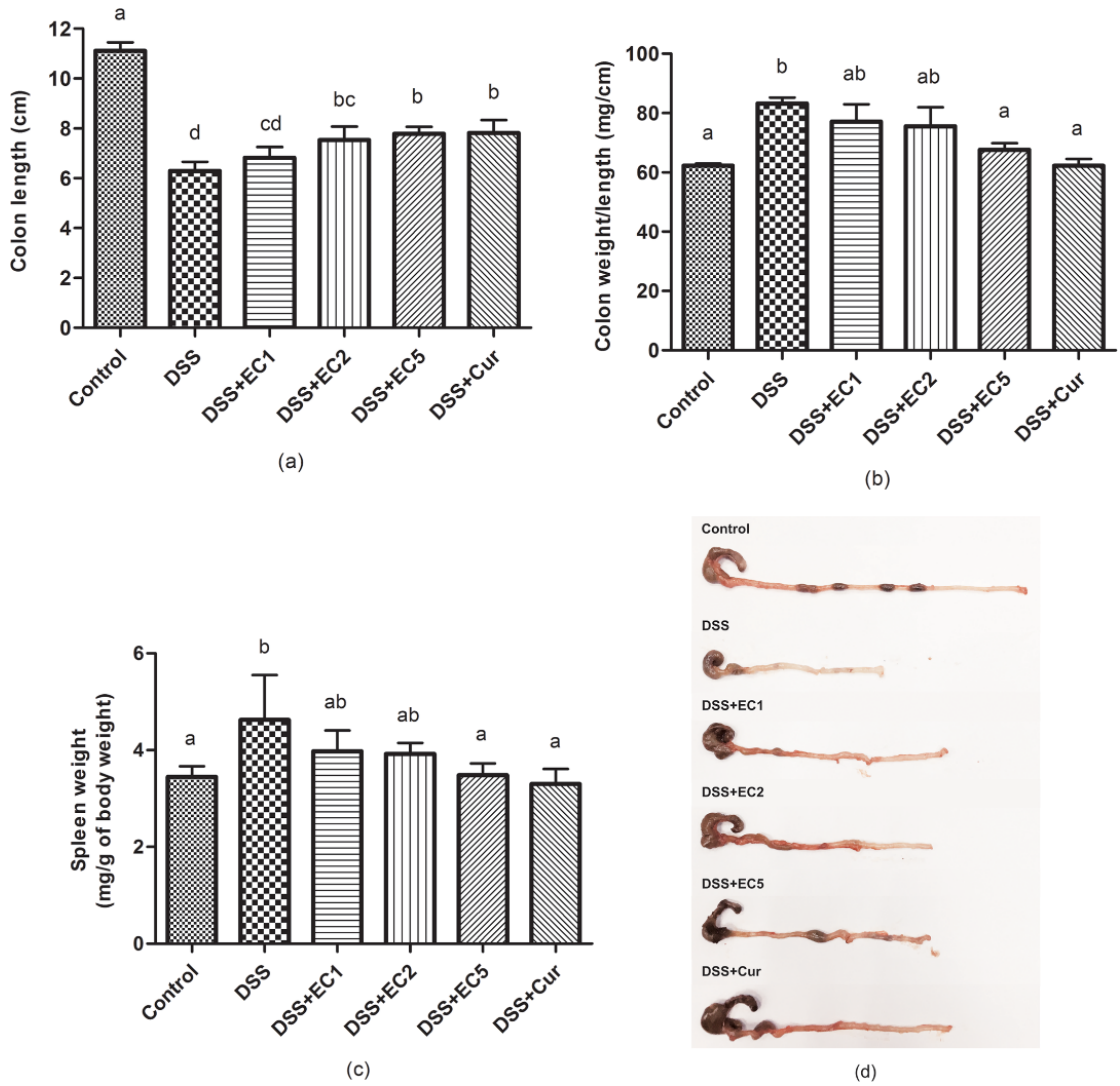


Fig 4. Effects of EC extract treatment on colon health after 7 days of treatment: (a) colon length, (b) colon weight/length ratio, (c) splenic weight, (d) representative colons for each group. Data are shown as the mean ± S.D. (n = 8). Letters indicate statistical significance (P <0.05) as analyzed by Duncan's multiple range test.

(Sudirman *et al.*, 2018)

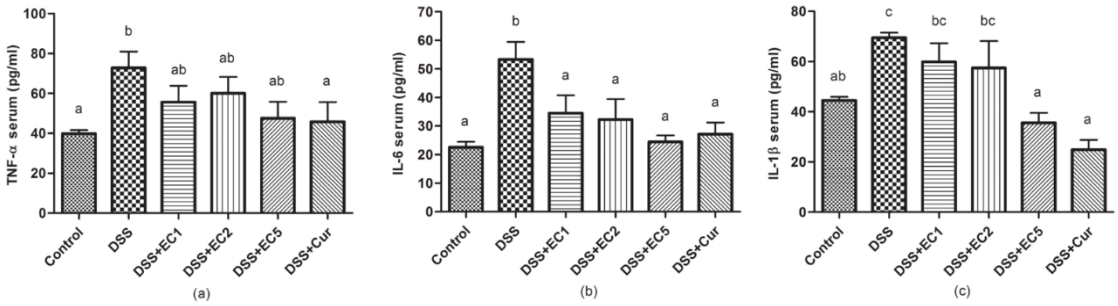


Fig 5. Effects of EC extract on inflammatory cytokine expression in serum after 7 days of treatment: (a) TNF-α, (b) IL-6, and (c) IL-1β. Data are shown as the mean ± S.D. (n = 8). Letters indicate statistical significance (P<0.05) as analyzed by Duncan's multiple range test.

(Sudirman *et al*., 2018)

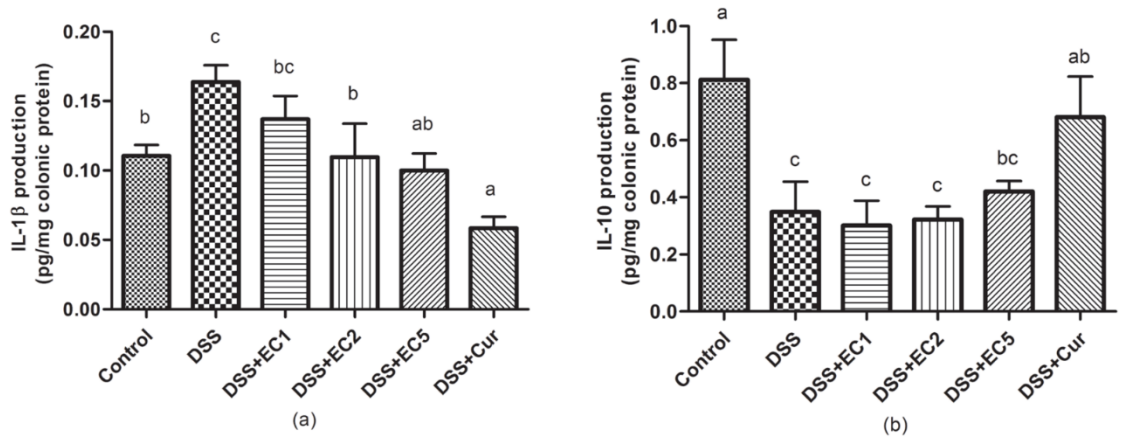


Fig 6. Effects of EC extract on inflammatory cytokine expression in colon tissue after 7 days of treatment: (a) IL-1β and (b) IL-10. Data are shown as the mean ± S.D. (n = 8). Letters indicate statistical significance (P<0.05) as analyzed by Duncan's multiple range test.

(Sudirman *et al*., 2018)

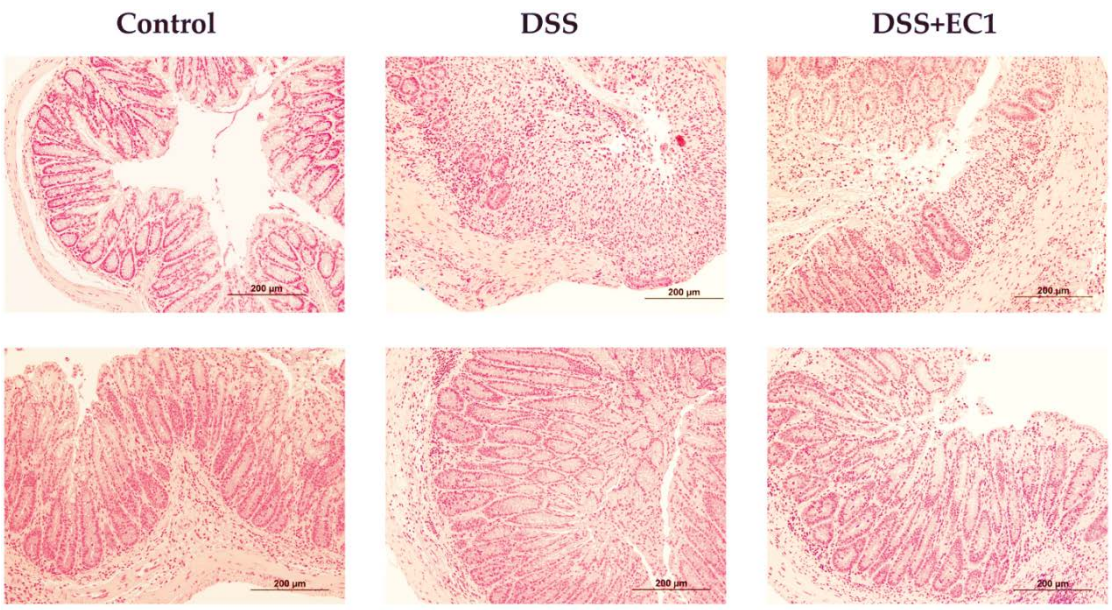


Fig 7. Representative colonic tissue histopathology for each group after 7 days of treatment.

(Sudirman *et al*., 2018)

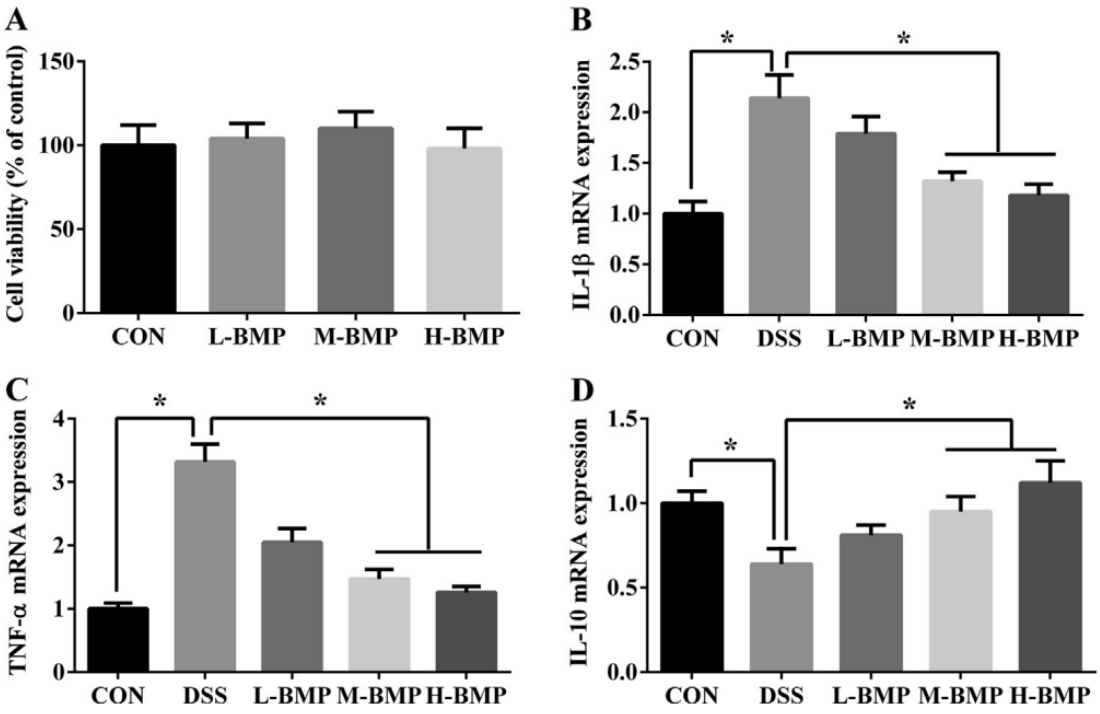


Fig. 8. Anti-inflammatory effects of BMP on IPEC-J2 cells. (A) Cell viability of control cells.

(Song *et al.*, 2019)

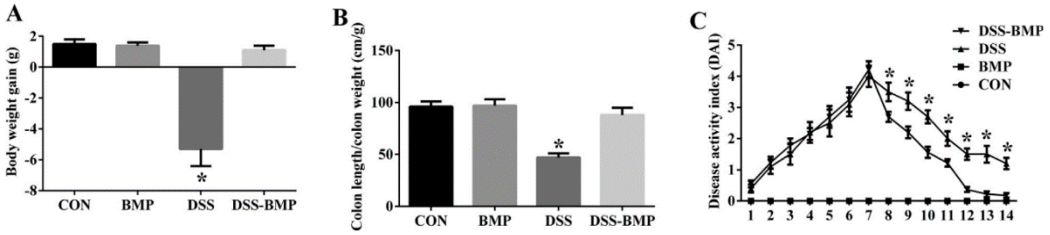


Fig. 9. BMP alleviates colitis symptoms. (A) Body weight loss; (B) Colon length/colon weight; (C) Disease activity index. CON, control mice; BMP, control mice supplemented with BMP; DSS, mice treated with dextran sulfate sodium; DSS-BMP, mice treated with dextran sulfate sodium and then supplemented with BMP. Values are expressed as mean ± SEM, n = 8. ∗Mean values were significantly different from all the other groups (P < 0.05).

(Song *et al.*, 2019)

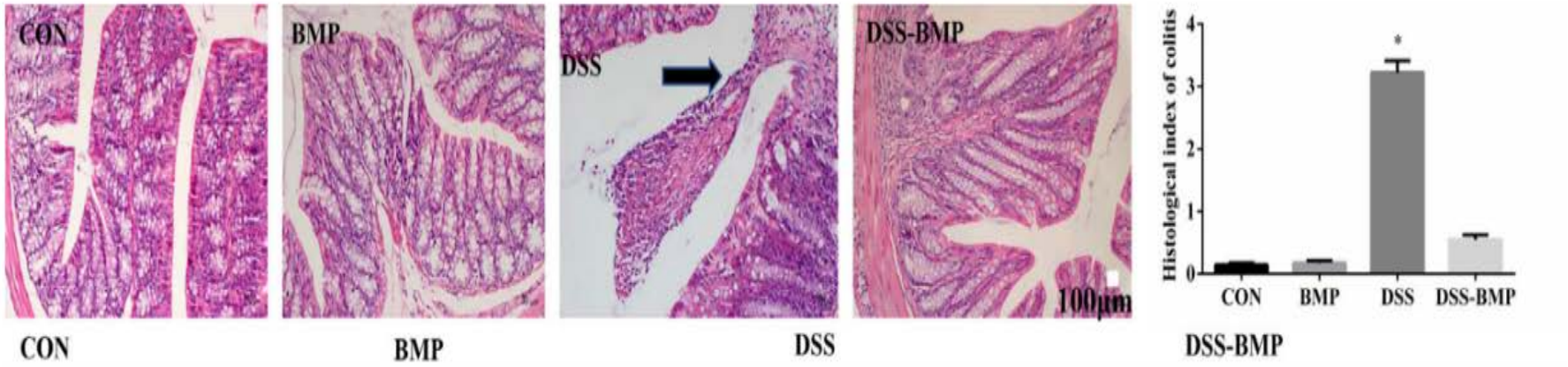


Fig. 10. BMP alleviates DSS-induced histopathological changes. (Upper) HE staining of colonic morphology (arrow, partial loss and sloughing of the villi). (Lower) ultrastructural observation of colon (transmission electron microscopy). CON, control mice; BMP, control mice supplemented with BMP; DSS, mice treated with dextran sulfate sodium; DSS-BMP, mice treated with dextran sulfate sodium and then supplemented with BMP. Values are expressed as mean ± SEM, n = 8. ∗Mean values were significantly different from all the other groups ($P < 0.05$).

(Song *et al.*, 2019)

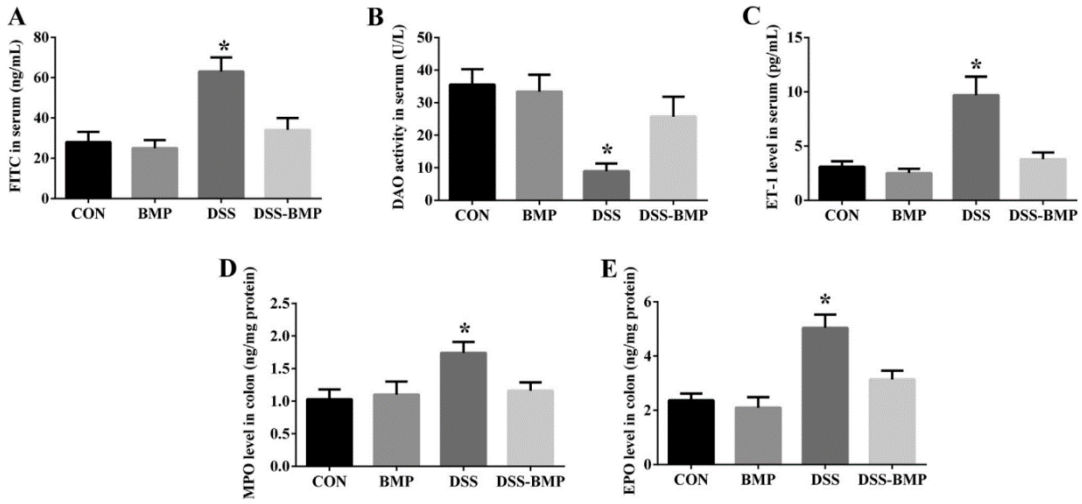


Fig. 11. BMP alleviates DSS-induced colonic infiltration and improves intestinal barrier function. (A) FITC in serum; (B) DAO activity in serum; (C) ET-1 level in serum; (D) MPO level in colon; (E) EPO level in colon. CON, control mice; BMP, control mice supplemented with BMP; DSS, mice treated with dextran sulfate sodium; DSS-BMP, mice treated with dextran sulfate sodium and then supplemented with BMP. DAO, diamine oxidase; EPO, eosinophil peroxidase; ET-1, endothelin 1; MPO, myeloperoxidase. Values are expressed as mean ± SEM, n = 8. ∗Mean values were significantly different from all the other groups ($P < 0.05$).

(Song *et al.*, 2019)

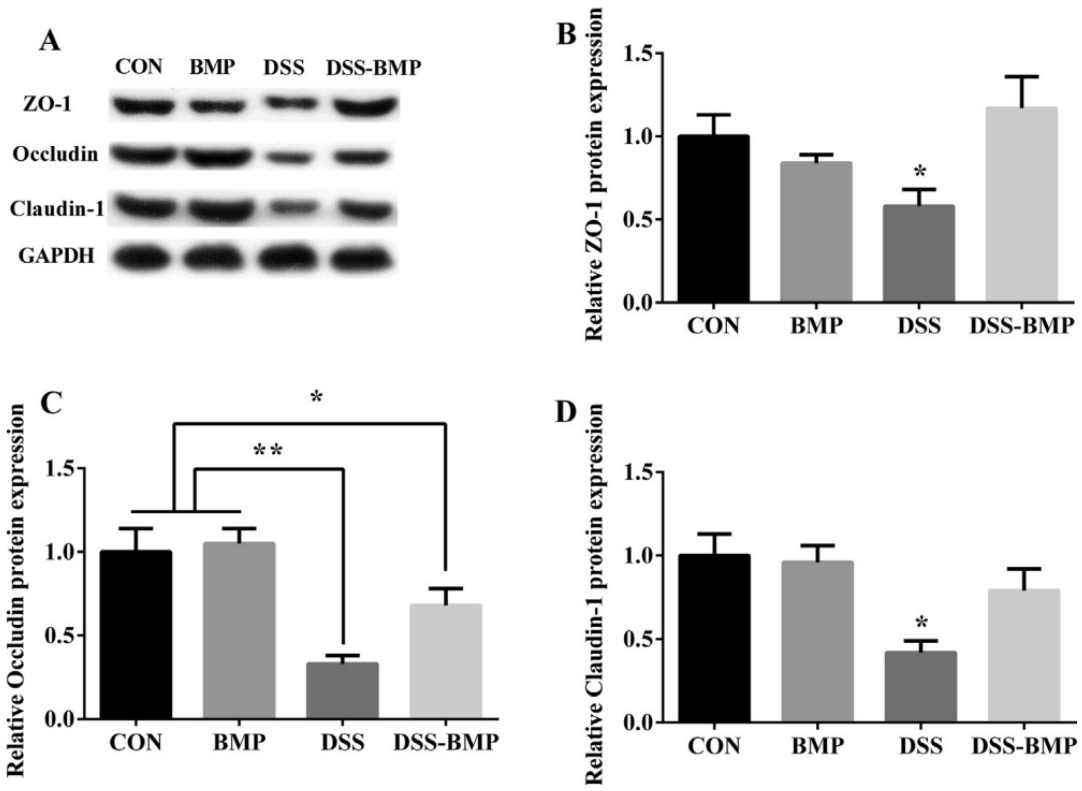


Fig. 12. BMP alleviates DSS-induced inhibition of tight junction protein expression. (A) Western bolting results. (B) Relative abundance of ZO-1. (C) Relative abundance of occludin. (D) Relative abundance of claudin-1. CON, control mice; BMP, control mice supplemented with BMP; DSS, mice treated with dextran sulfate sodium; DSS-BMP, mice treated with dextran sulfate sodium and then supplemented with BMP. Values are expressed as mean ± SEM, n = 3. ∗Mean values were significantly different among groups (P < 0.05); **Mean values were significantlydifferent among groups (P < 0.01).

(Song *et al.*, 2019)

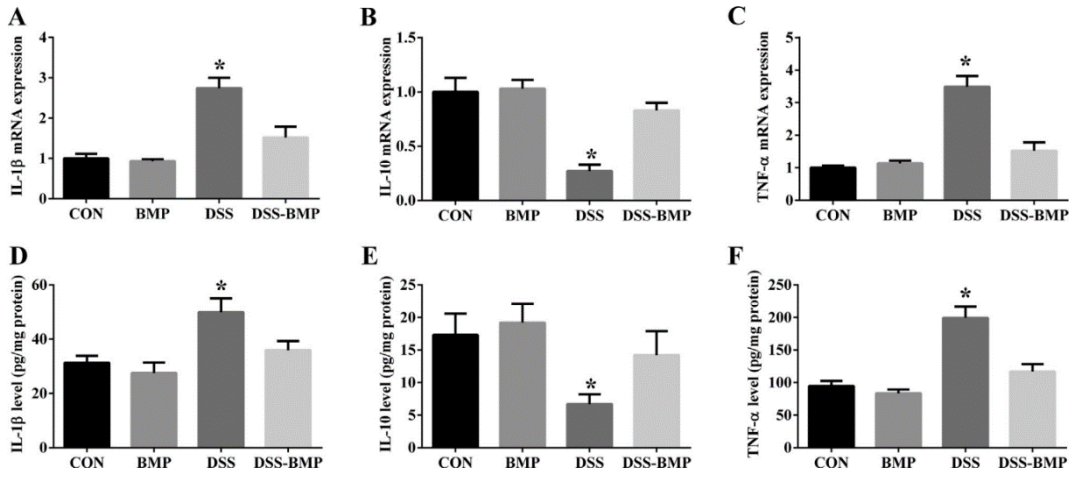


Fig. 13. BMP alleviates DSS-induced inflammatory response. mRNA expression of IL-1β (A), IL-10 (B) and TNF-α (C) in colon; Concentration of IL-1β (D), IL-10 (E) and TNF-α (F) in colon. CON, control mice; BMP, control mice supplemented with BMP; DSS, mice treated with dextran sulfate sodium; DSS-BMP, mice treated with dextran sulfate sodium and then supplemented with BMP. IL-1β, interleukin 1β; IL-10, interleukin 10; TNF-α, tumor necrosis factor α. Values are expressed as mean ± SEM, n = 8. ∗ Mean values were significantly different from all the other groups ($P < 0.05$).

(Song *et al.*, 2019)

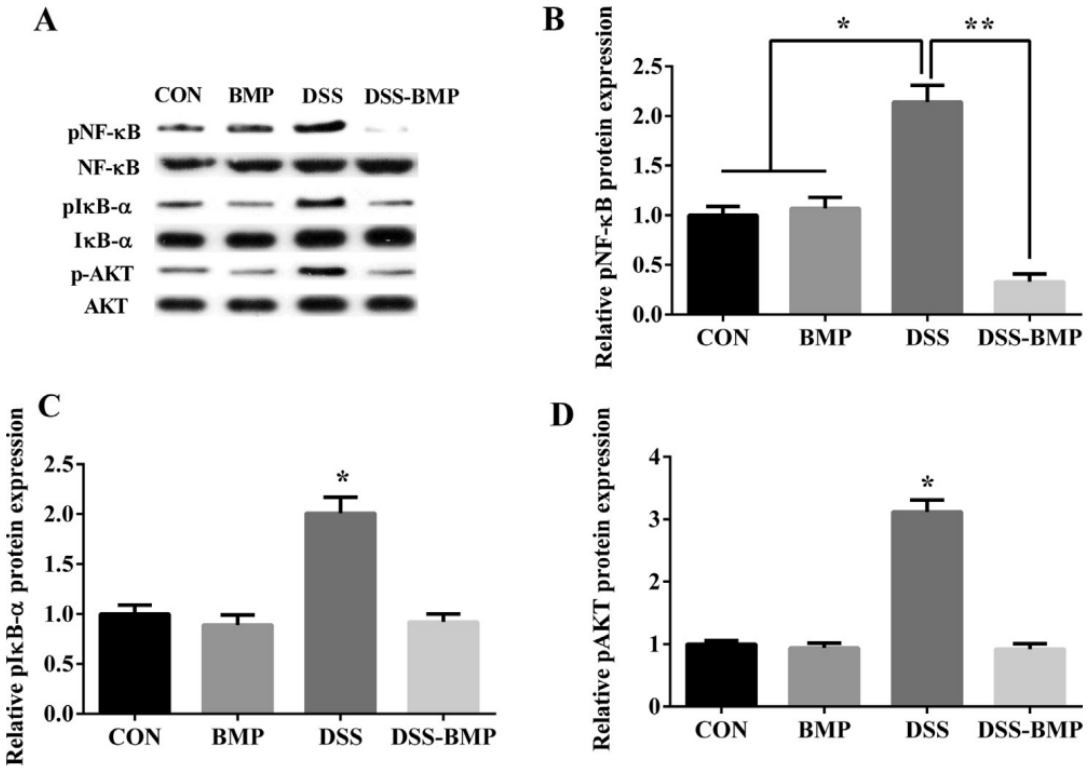


Fig. 14. BMP alleviates DSS-induced activation of NFκB and AKT pathway. (A) Western bolting results. (B) Relative abundance of phosphorylated NFκB. (C) Relative abundance of phosphorylated IκB-α. (D) Relative abundance of phosphorylated AKT. CON, control mice; BMP, control mice supplemented with BMP; DSS, mice treated with dextran sulfate sodium; DSS-BMP, mice treated with dextran sulfate sodium and then supplemented with BMP. Values are expressed as mean ± SEM, n = 3. ∗Mean values were significantly different among groups (P < 0.05); **Mean values were significantly different among groups (P < 0.01).

(Song *et al.*, 2019)

Table 1. Disease activity index (DAI) scoring of DSS-induced colitis.

Score	Occult/gross bleeding	Weight loss (% of initial weight)	Stool consistency
0	None	< 1	Normal stools
1	Small spots of blood stool; dry anal region	1–5	Soft pellets not adhering to the anus
2	Large spots of blood in stool; blood appears through the anal orifice	5–10	Very soft pellets adhering to the anus
3	Deep red stool; blood spreads largely around the anus	10–15	Liquid stool in long streams; wet anus
4	Gross bleeding	> 15	Diarrhea

Table adapted from previous studies [35, 40, 41].

(Sudirman *et al.*, 2018)