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Original Research Article

The Effect of Pomegranate Peel (*Punica granatum* L.) Extract in Chitosan Nanoparticle on the Macrophage Polarization in DSS-Induced Mice

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ABSTRACT

Macrophage polarization towards type 1 (M1) or type 2 (M2) is a critical determinant in re-establishing inflammation within the colon. Pomegranate is widely recognized as a biological source for its substantial quantity and potent anti-inflammatory properties. This investigation compares the effects of chitosan nanoparticles infused with pomegranate peel extract (*Punica granatum* L.) on the polarization of macrophages in mice's peritoneal fluid induced with dextran sodium sulfate (DSS). In this study, the male Balb/c mice were aged 12-16 weeks and had a body weight of 20-30 g. Randomly, six distinct groups of Balb/c mice were established. A total of 42 days of treatment, or 3 × 14 days, were administered in six treatment groups. After the experiment, the quantity of M1 and M2 macrophages in the peritoneal fluid was quantified through flow cytometric analysis. After administering 240 mg/kg chitosan-PPE nanoparticles (10.57±0.51%) increase in M2 macrophages was observed ($p<0.05$). Upon administration of 480 mg/kg chitosan-PPE nanoparticles (P2 groups) and unadulterated PPE at 480 mg/kg doses (P3 groups) and comparison to the DSS groups, a substantial increase in M2 macrophages (11.20±2.10%) and P3 groups (17.85±0.44%) was observed. In contrast, a concurrent decrease in M1 macrophages (67.23±2.18%) and P3 groups (60.33±8.42%) was observed ($p<0.05$). These results support the evidence notion that pomegranate peel extract has a significant anti-inflammatory effect and influences the polarization of macrophages.

Keywords: Chitosan nanoparticles, Colon inflammation, Macrophages, Pomegranate peel extract.

Introduction

Colon inflammation is a commonly occurring inflammatory disorder that impacts the region of the colon. This inflammation's acute or chronic status is established according to the duration of the pathogenic agent's presence and the immune system's response to the inflammation.[1] Chronic inflammation, such as ulcerative colitis (UC), may manifest as inflammatory bowel disease (IBD).[2] Chronic UC may lead to an elevated risk of developing colorectal cancer.[3] The incidence rates corresponded to cumulative probabilities of 2% by 10 years and increase 18% at 30 years.[3] However, a subsequent meta-analysis conducted over a decade later indicated that the incidence of colorectal cancer (CRC) in people with IBD has diminished, with rates of 1%, 2%, and 5% after 10, 20, and more than 20 years of disease duration, respectively.[4] This meta-analysis should be interpreted cautiously due to including diverse and heterogeneous studies. In contrast to the general population, the rates of CRC among IBD patients are still higher even though they are consistently decreasing. Special consideration must be given to patients with IBD, particularly those who have risk factors, during their follow-up for CRC.

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In developing animal models of UC, dextran sodium sulfate (DSS) is frequently employed as an induction agent.[5] The most severe murine colitis, resembling human UC, results from administering 40-50kDa DSS in drinking water.[6] The administration of DSS, a sulfated polysaccharide toxic to the colonic epithelium, disrupts the permeability of epithelial cells and can induce an immune response, thereby disrupting the mucosal barrier throughout the colonic epithelium.[7] DSS does not directly induce intestinal inflammation; it disrupts the intestinal epithelium's barrier, exposing the lamina propria (LP) to the external environment and allowing luminal antigens and microbes to enter and induce inflammation.[6] Numerous variables, including the dose (typically 1-5%), the duration (acute or chronic), the animal's strain, the animal's sex (male rats are more susceptible), and the animal's microbial environment (e.g., germ-free [GF] or specific pathogen-free [SPF]), have an impact on the efficacy of DSS-induced colitis.[8] Because of this, DSS is extensively employed in the study of the immunological processes associated with the development of intestinal inflammation.

In both adaptive and innate immunity, macrophages are indispensable sentinels that undergo a series of functional activation states in response to specific homeostatic and pathological conditions.[9] Activated macrophages display antimicrobial, phagocytic, and pro-inflammatory properties in reaction to stimuli within their immediate microenvironment. Additionally, they may contribute to the resolution of inflammation, T cell suppression, and tissue remodeling.[10] Macrophages are categorised into two types: M1 macrophages, which are traditionally activated by interferon (IFN)-γ or lipopolysaccharides (LPS), and M2 macrophages, which are alternatively triggered by interleukin (IL)-4 or IL-10. M2 macrophages can be subdivided into M2a, M2b, and M2c subtypes, each characterised by distinct biological roles, surface markers, and secretory cytokines.[11] Modulating the functions of distinct phenotypes may offer a promising therapy for

diseases in which these macrophages are involved, as they perform various functions.

The extraordinarily diverse flora and fauna of Indonesia hold immense ethnomedical application potential. Pomegranate is widely acknowledged as a biological source renowned for its prodigious quantities and anti-inflammatory attributes. In prior investigations, we discovered that pomegranate extract ethanol could stimulate apoptosis by upregulating the expression of Caspase-3,[12] inhibiting the expression of COX-2 and iNOS,[13] and reducing the expression of TNF-α in mice induced with DSS.[14] The byproduct of ellagitannin hydrolysis in the intestine is ellagitannin acid.[15] Pomegranate peel contains 13% w/w ellagic acid, an anti-inflammatory, anti-bacterial, and anti-allergic compound. Research conducted *in vivo* has shown that ellagic acid can reduce inflammation in the colon caused by DSS.[16] Ellagic acid inhibits the NF-κB inflammatory signaling pathway, impedes intestinal inflammation, and suppresses the expression of COX-2 and iNOS.[17] When administered to mice for a period of four weeks at a daily dose of 6 mg, pomegranate peel extract inhibited the expression of COX-2 in both the colon and visceral adipose tissue.[17] Pomegranate peel is also abundant in punicalagin (PUN), a compound that is derived from ellagitannin and extensively documented for the treating inflammatory diseases.[18,19]

In this study, we reported the comparative effects of chitosan nanoparticles-PPE and pomegranate peel extract (PPE) on macrophage polarization in the peritoneal fluid of DSS-induced mice. Our discovery offered novel and significant evidence to enhance comprehension of the activities of pomegranate peel extract and the underlying mechanism by which it induces macrophage polarization in inflammatory diseases.

Materials and Methods

Sample and Study Design

The male Balb/c mice in this *in vivo* investigation were between 12 and 16 weeks old and weighed between 20-30 g.[20] Breeding for the mice took place at the Kemuning Karya International in Central Java, Indonesia. The experimental setup comprised a standard laboratory setting at the Department of Anatomical Pathology Animal House facilities, wherein the temperature was maintained at 22 °C and the relative humidity was 65%. Water and standard mice pellets were supplied freely to the mice during the treatment. Based on Federer's formula, the minimum number of mice in a single group is four. Consequently, the total number of mice (n) is 24. Considering the sample attrition during the study, the minimum number of samples was upped by 10% from the initial minimum number to 27 mice. The Universitas Indonesia Institutional Animal Ethics Committee granted this experiment ethical approval on 7th February 2022, by protocol number KET-114/UN2.F1/ETIK/PPM.00.02/2022.

Drugs and Chemicals

Pomegranate peel (*Punica granatum* Linn.) was obtained from the Conservation Unit of the IPB Biopharmaceutical Garden. Pomegranate peel was extracted and macerated in the laboratory of the IPB Biopharmaceutical Study Center. Ellagic acid (EA) with 338.20kDa was purchased from Santa Cruz Biotechnology (Dallas, TX, USA) with purity ≥96%. DSS with a molecular weight of 60kDa was purchased from Sigma-Aldrich (St. Louis, MO, USA). The antibody for flow cytometry analysis was purchase from BioLegend (San Diego, CA, USA) consisting of APC anti-mouse CD68 antibody, PerCP/Cyianine5.5 anti-mouse CD25 antibody, PE anti-mouse CD80 antibody, and FITC anti-mouse CD206 (MMR). The dosage calculation utilized in this experiment was based on previous research.[13] The dosage of purified ellagic acid was ascertained through a conversion process using the dosage specified by the Food and Drug Administration (FDA) of the United States, which is 26 mg/kg/day. The value of 240 mg/kg/day for the abundance level of polyphenols in pomegranates was utilized as the standard for determining the dosage of nanoparticle chitosan-PPE in this investigation. Utilizing both, 480 mg/kg/day was the second dose of pomegranate peel extract, which was determined by geometric sequence.

Experimental Design

Over a period of 3 × 14 days (42 days), the investigation was conducted in six treatment groups. Oral administration of all treatments was accomplished through the consumption of beverages. Throughout the investigation, mice in the normal group were administered water orally. All other groups were administered water containing 2% DSS for a period of seven days at the commencement of the second and fourth phases. For a period of seven days, the DSS was administered at regular intervals of water consumption.[12] The negative control group was not given any other treatment other than DSS. Two groups were given nanoparticle chitosan-PPE in two different doses (480 mg/kg/day and 240 mg/kg/day) for each group at the start of the first week of the experiment. One group was given PPE doses of 480 mg/kg/day. Pure ellagic acid was given to the positive control groups at the start of the first week after DSS induction. All the groups of mice were sacrificed three days after the last treatment of DSS to obtain the peritoneal fluid.

Flow Cytometry Analysis

The samples used for macrophage polarization analysis from peritoneal fluid were taken from each group of experimental animals by adding 0.9% NaCl solution. Then, the peritoneal solution was centrifuged (Thermo scientific, Sorvall Legend, XTR) at 2500 rpm for 5 minutes.[21] After centrifugation, two phases were formed, namely pellets and supernatant. The pellet was separated from the supernatant; then, the pellet was resuspended with buffer stains 1mL. Continuously, 200 µL of the suspension was incubated with antibodies CD25 (PercP Cy5.5), CD206 (FITC), CD80 (PE), and CD68 (APC) for 30 minutes. After incubation, the suspension was washed twice using buffer stains. Furthermore, the sample is read using the flow cytometer instrument (BD FACSCanto II, BioScience, New Jersey, USA). The flow cytometry test was conducted twice for each sample to ensure its repeatability.

Statistical Analysis

The primary table was prepopulated with data that was collected using Microsoft Excel prior to the analysis (Microsoft Corp, Redmond, WA, USA). Statistical Package for the Social Sciences (SPSS) Version 20 (IBM Corp, Armonk, NY, USA) was employed to conduct the visualisation and analysis. The resulting data were verified for normal distribution and homogeneity using the Shapiro-Wilk and Levene tests, which required that the data be normal and homogeneous.[13] For each treatment, the data is represented by the mean by standard deviation (SD) values. Additionally, Data were subjected to a one-way ANOVA test, which was followed by a Tukey post-hoc analysis. The Kruskal-Wallis and Mann-Whitney tests were implemented to evaluate groups that were not normally distributed and homogeneous datasets. A statistically significant p-value was established at $p < 0.05$.

Results and Discussion

Pomegranate peel extract decrease M1 macrophage population

Leukocytes are initially separated utilizing $CD45^+$ antibodies before sorting macrophages. Applying $CD68^+$ antibodies to the resident monocyte (M0) and macrophage lines, the $CD45^-$labeled leukocyte population was sorted. By selecting $CD68^+$ and $CD80^+$ markers, the number of M1 macrophages was determined; these markers were quantified using flow cytometry on the percentage population of M1 macrophages. The flow cytometry analysis yielded the following results regarding the presence of M1 macrophage cells ($CD68^+CD80^+$) in the peritoneal fluid of Balb/c mice model of colon inflammation: normal group (62.90±9.01%), ellagic acid group(81.55±1.60%), negative control or DSS group (74.85±4.54%), pomegranate extract nanoparticles dose 480 mg/kg (P1 group) (83.43±3.86%), pomegranate extract nanoparticles dose 240 mg/kg (P2 group) (67.23±2.18%), and pomegranate extract dose 480 mg/kg (P3 group) (60.33±8.42%) (Figure 1). The statistical analysis revealed a noteworthy reduction ($p<0.05$) in the quantity of M1 macrophages in group P3 and group P2 compared to the negative control group.

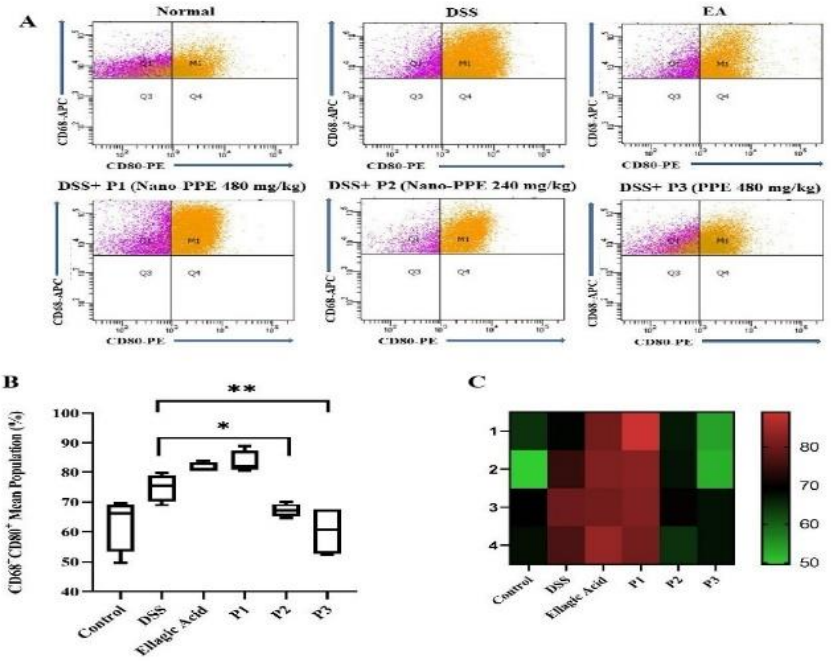

Figure 1: PPE decreased macrophage M1 population in DSS-induced mice colitis model. A. Proportion of $CD68^{+}CD80^{+}$ macrophage M1 in $CD68^{+}$ macrophage M0. B. The outcomes of examining the impact of pomegranate peel extract on the polarization of M1 macrophages. Each bar represents the mean ± SD of the M1 population. C. The consistency of each replication is illustrated in the heatmap graph by showing the response of each mouse in the same group to the treatment received. *There was a significant difference between negative control and P2 (PPE-loaded in chitosan nanoparticles at a dose of 240 mg/kg) group. **There was a significant difference between negative control and pomegranate peel extract at 480 mg/kg (P3).

The analysis results indicated a substantial reduction in M1 macrophages in the treatment group after applying PPE at 480 mg/kg. This decline constituted a 14.5% reduction in the number of M1 macrophages when compared to the DSS control group. Our previous research results also support the notion that punicalagin, which is present in PPE, inhibits the proliferation of M1 macrophages as measured by decreased mRNA levels of IL-1, IL-6, and TNF-α.[22–24]

Th1 cells are secreted IL-1, IL-6, and TNF-α, alongside M1 macrophages and dendritic cells. In colonic tissue, these three cytokines contribute to developing an inflammatory response.[15] TNF-α is produced by mononuclear cells and is responsible for promoting inflammation via various mechanisms.[25] These include fibroblast proliferation, activation of procoagulant factors, initiation of an acute response, and fibroblast proliferative responses to adhesion molecules.[26] In addition to stimulating B cells and activating macrophages and lymphocytes, TNF-α also increases the production of IFN-α by T cells. The proinflammatory and regulatory functions of IL-1, produced by macrophages, are involved in the pathogenesis of ulcerative colitis.[27] The primary products of IL-1 are IL-1α and IL-1β, which act as stimulants for phospholipase A, iNOS, and COX-2. IL-1α receptor antagonists (IL-1Ra) function as a mechanism of regulation.[28]

Significantly fewer M1 macrophages were present in the P1 treatment group due to 480 mg/kg of chitosan nanoparticle-encapsulated pomegranate peel extract compared to the DSS control. Notably, the P2 group administered 240 mg/kg of chitosan nanoparticle-encapsulated pomegranate peel extract but did not encounter this consequence. Hence, it is established that the formulation of chitosan nanoparticle-encapsulated pomegranate peel extract at a concentration of 480 mg/kg significantly inhibits the proliferation of M1 macrophages. In contrast, the quantity of M1 macrophages increased in the positive control group (ellagic acid group). As a result, this inquiry implies that ellagic acid cannot directly inhibit the population of M1 macrophages. The latest study discovered that ellagic acid significantly inhibited the proliferation of M1 macrophages in the form of derivatives of the urolithin A metabolism of small intestine microbiota.[29]

Administration of pomegranate peel extract augments the number of M2 macrophages

$CD206^{+}$ and $CD68^{+}$ are the specific markers utilized to distinguish M2 macrophages. The analysis of M2 macrophage ($CD68^{+}CD206^{+}$) proportions yielded the following results: normal group (10.23±0.70%), DSS group (7.30±0.29%), EA group (6.30±0.52%), P1 group (10.58±1.01%), P2 group (11.20±2.10%), and P3 group (17.85±0.44%) (Figure 2). A statistically significant increase ($p<0.05$) in M2 macrophage populations was observed in the P1, P2, and P3 groups compared to the DSS group. The flow cytometry analysis revealed that the quantity of M2 macrophages in the group treated with 480 mg/kg PPE increased by 10.57%±0.51% compared to the group treated with DSS. Additionally, compared to the DSS control group, the number of M2 macrophages increased by 3.27% and 3.90%, respectively, in the P1 group (PPE-loaded in chitosan nanoparticles at a dose of 480 mg/kg) and P2 group (PPE-loaded in chitosan nanoparticles at a dose of 240 mg/kg). According to these results, three doses of PPE and PPE encapsulated in chitosan nanoparticles significantly increased the number of peritoneal M2 macrophages in mice induced with 2% DSS. These findings in line with research that has demonstrated that the level of Arg I (M2) expression can be preserved at the same level as that in the aorta of juvenile mice through the administration of pomegranate extract.[22] In addition, it is recognised that punicalagin, one of the active compounds in pomegranate peel extract, has the capacity to elevate the expression of Arg-I, a marker for M2 macrophages, and IL-10 cytokines.[31]

The enhanced expression of Arg-1 and IL-10 was utilized to determine these outcomes. IL-10 is secreted by B cells, T cells, and monocytes to reduce the antigen-presenting capability of monocytes, thereby inhibiting the production of TNF-α, IL-1, and IL-6. Interleukins-10 functions as a protective agent within the mucosal immune system.[32] Inhibiting IL-10 increases the production of IL-12 and IFN-γ in mice with chronic colitis.[33] Due to the absence of IL-10 signaling, a correlation has been identified between IL-10 receptor mutations and the severity of colitis. [34] Furthermore, immune dysregulation has been

linked to IL-10 A and B receptor subunits (IL-10RA and IL-10RB) mutations in patients with colitis.[35]

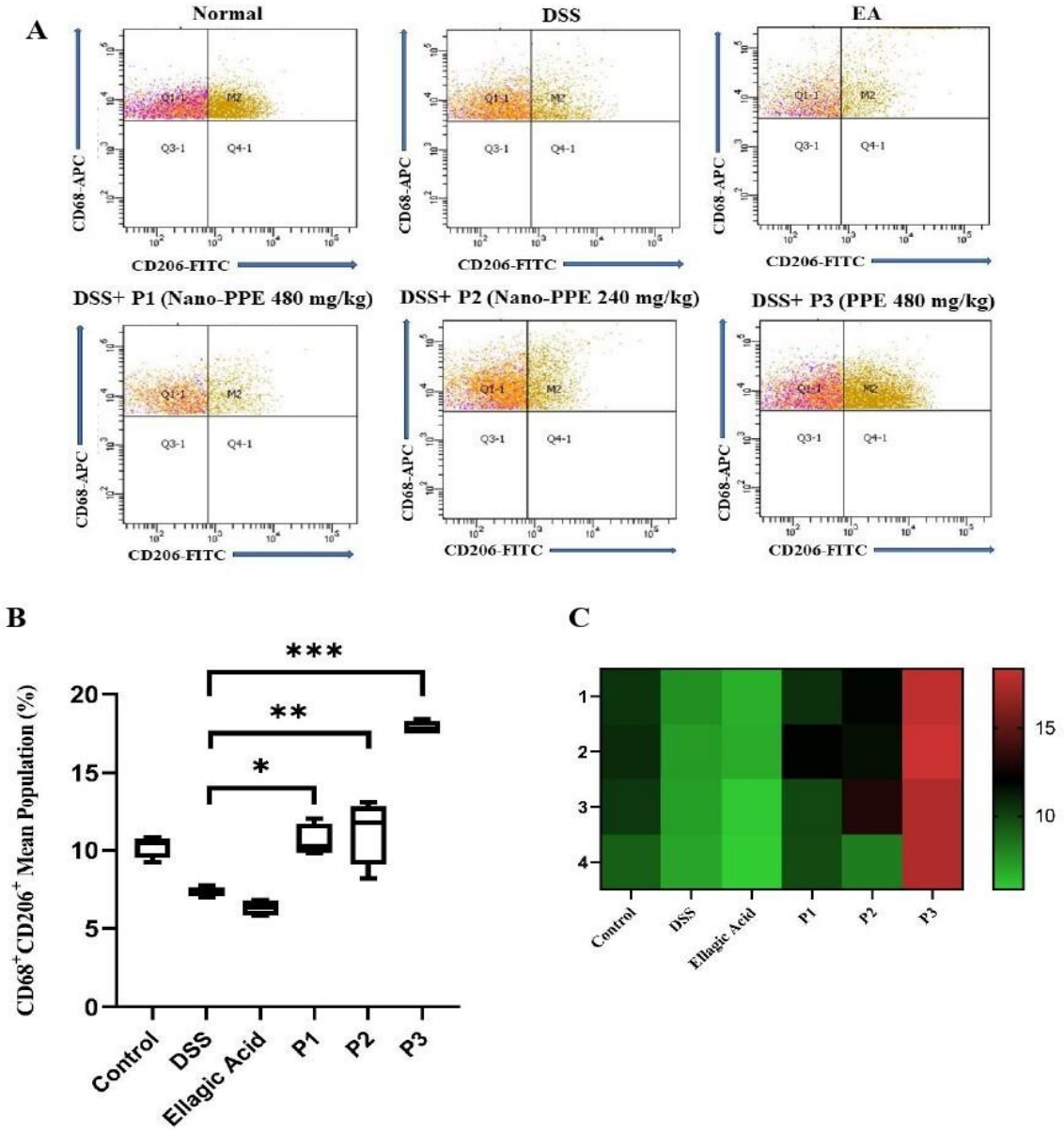

Figure 2: PPE increased macrophage M2 population in DSS-induced mice colitis model. A. Proportion of $CD68^{+}CD206^{+}$ macrophage M2 in $CD68^{+}$ macrophage M0. B. analysis results on the impact of pomegranate peel extract on the polarization of M2 macrophages. Each bar represents the mean ± SD of the M2 population. C. The heatmap graph illustrates that each replication is consistent by showing the response of each mouse in the same group to the received treatment. *There was a significant difference between negative control and PPE-loaded chitosan nanoparticles at a dose of 480mg/kg (P1). **There was a significant difference between negative control and PPE-loaded chitosan nanoparticles at a dose of 240 mg/kg (P2). ***There was a significant difference between negative control and pomegranate peel extract at 480 mg/kg (P3).

Conclusion

Chitosan-PPE nanoparticles at a 240 mg/kg dose increased M2 macrophages but did not significantly reduce M1 macrophages. In contrast, both chitosan-PPE nanoparticles and pure PPE at a dose of 480 mg/kg significantly decreased M1 macrophages while increasing M2 macrophages. These results suggest that pomegranate peel extract, particularly at higher doses and in nanoparticle formulations, holds potential as a modulator of macrophage polarization with notable anti-inflammatory properties. Further research will be conducted to examine the regulatory effects of punicalagin and the crude extract of pomegranate rind on other immune cells.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Mowat AMI, Bain CC. Mucosal macrophages in intestinal homeostasis and inflammation. J Innate Immun. 2011; 3(6):550–564. Doi: 10.1159/000329099
2. Tatiya-Aphiradee N, Chatuphonprasert W, Jarukamjorn K. Immune response and inflammatory pathway of ulcerative colitis. J Basic Clin Physiol Pharmacol. 2018; 30(1):1-10. Doi: 10.1515/jbcpp-2018-0036
3. Gajendran M, Loganathan P, Jimenez G, Catinella AP, Ng N, Umapathy C, Ziade N, Hashash JG. A comprehensive review and update on ulcerative colitis. Dis Mon. 2019; 65(12):1-37. Doi: 10.1016/j.disamonth.2019.02.004
4. Lutgens MWMD, van Oijen MGH, van der Heijden GJMG, Vleggaar FP, Siersema PD, Oldenburg B. Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. Inflamm Bowel Dis. 2013; 19(4):789–799. Doi: 10.1097/mib.0b013e31828029c0
5. Bilsborough J, Fiorino MF, Henkle BW. Select animal models of colitis and their value in predicting clinical efficacy of biological therapies in ulcerative colitis. Expert Opin Drug Discov. 2021; 16(5):567–577. Doi: 10.1080/17460441.2021.1851185
6. Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. Curr Protoc Immunol. 2014; 104(15):1-14. Doi: 10.1002/0471142735.im1525s104
7. Perše M, Cerar A. Dextran sodium sulphate colitis mouse model: Traps and tricks. J Biomed Biotechnol. 2012; 2012:1-13. Doi: 10.1155/2012/718617
8. Low D, Nguyen DD, Mizoguchi E. Animal models of ulcerative colitis and their application in drug research. Drug Des Devel Ther. 2013; 7:1341–1356. Doi: 10.2147/dddt.s40107
9. Galli SJ, Borregaard N, Wynn TA. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. Nat Immunol. 2011; 12(11):1035–1044. Doi: 10.1038/ni.2109
10. Lissner D, Schumann M, Batra A, Kredel LI, Kühl AA, Erben U, May C, Schulzke JD, Siegmund B. Monocyte and M1 Macrophage-induced Barrier Defect Contributes to Chronic Intestinal Inflammation in IBD. Inflamm Bowel Dis. 2015; 21(6):1297-1305. Doi: 10.1097/mib.0000000000000384
11. Lisi L, Stigliano E, Lauriola L, Navarra P, Dello Russo C. Proinflammatory-activated glioma cells induce a switch in microglial polarization and activation status, from a predominant M2b phenotype to a mixture of M1 and M2a/B polarized cells. ASN Neuro. 2014; 6(3):171–183. Doi: 10.1042/an20130045
12. Kusmardi K, Azzahra Baihaqi L, Estuningtyas A, Sahar N, Sunaryo H, Tedjo A. Ethanol Extract of Pomegranate (*Punica granatum*) Peel in Increasing the Expression of Caspase-3 in DSS-Induced Mice. Int J Inflam. 2021; 2021:1-7. Doi: 10.1155/2021/4919410
13. Kusmardi K, Hermanto D, Estuningytas A, Tedjo A, Priosoeryanto BP. The potency of Indonesia's pomegranate peel ethanol extract (*Punica granatum* Linn.) as anti-inflammatory agent in mice colon induced by dextran sodium sulfate: Focus on cyclooxygenase-2 and iNOS expressions. Asian J Pharm Clin Res. 2017; 10(12):370–375. Doi: 10.22159/ajpcr.2017.v10i12.21390
14. Kusmardi K, Yasmin Khalilah R, Zuraidah E, Estuningtyas A, Tedjo A. The Effect of Pomegranate Peel Ethanol Extract on TNF-α Expression of Mice Colonic Epithelial Cells Induced Using Dextran Sodium Sulfate (DSS). Phcog J. 2022; 14(3):480–488. Doi: 10.5530/pj.2022.14.61
15. Abd El-Rady NM, Dahpy MA, Ahmed A, Elgamal DA, Hadiya S, Ahmed MAM, Sayed ZEA, Abdeltawab D, Abdelmohsen AS, Farrag AAM, Ashmawy AM, Khairallah MK, Galal HM. Interplay of Biochemical, Genetic, and Immunohistochemical Factors in the Etio-Pathogenesis of Gastric Ulcer in Rats: A Comparative Study of the Effect of Pomegranate Loaded Nanoparticles Versus Pomegranate Peel Extract. Front Physiol. 2021; 12:1–20. Doi: 10.3389/fphys.2021.649462
16. Rahaman MM, Rakib A, Mitra S, Tareq AM, Emran TB, Shahid-Ud-Daula AFM, Amin MN, Simal-Gandara J. The genus *Curcuma* and inflammation: Overview of the pharmacological perspectives. Plants. 2021; 10(1):1–19. Doi: 10.3390/plants10010063
17. Vučić V, Grabež M, Trchounian A, Arsić A. Composition and Potential Health Benefits of Pomegranate: A Review. Curr Pharm Des. 2019; 25(16):1817–1827. Doi: 10.2174/1381612825666190708183941
18. Velagapudi R, Baco G, Khela S, Okorji U, Olajide O. Pomegranate inhibits neuroinflammation and amyloidogenesis in IL-1β-stimulated SK-N-SH cells. Eur J Nutr. 2016; 55(4):1653–1660. Doi: 10.1007/s00394-015-0984-0
19. Zhao F, Pang W, Zhang Z, Zhao J, Wang X, Liu Y, Wang X, Feng Z, Zhang Y, Sun W, Liu J. Pomegranate extract and exercise provide additive benefits on improvement of immune function by inhibiting inflammation and oxidative stress in high-fat-diet-induced obesity in rats. J Nutr Biochem. 2016; 32:20-28. Doi: 10.1016/j.jnutbio.2016.02.003
20. Kusmardi K, Tumpu NA, Estuningtyas A. *Phaleria macrocarpa* leaf extract-chitosan nanoparticles supress angiogenesis induced by dextran sodium sulfate in mice colon. Int J Appl Pharm. 2019; 11(6):122–124. Doi: 10.22159/ijap.2019.v11s6.33574
21. Xu J, Zhao Y, Aisa HA. Anti-inflammatory effect of pomegranate flower in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages. Pharm Biol. 2017; 55(1):2095-2101. Doi: 10.1080/13880209.2017.1357737
22. Aharoni S, Lati Y, Aviram M, Fuhrman B. Pomegranate juice polyphenols induce a phenotypic switch in macrophage polarization favoring a M2 anti-inflammatory state. Biofactors. 2015; 41(1):44-51. Doi: 10.1002/biof.1199
23. Mastrogiovanni F, Mukhopadhya A, Lacetera N, Ryan MT, Romani A, Bernini R, Sweeney T. Anti-Inflammatory Effects of Pomegranate Peel Extracts on *In Vitro* Human Intestinal Caco-2 Cells and *Ex Vivo* Porcine Colonic Tissue Explants. Nutrients. 2019; 11(3):548. Doi: 10.3390/nu11030548
24. Čolić M, Bekić M, Tomić S, Đokić J, Radojević D, Šavikin K, Miljuš N, Marković M, Škrbić R. Immunomodulatory Properties of Pomegranate Peel Extract in a Model of Human Peripheral Blood Mononuclear Cell Culture. Pharmaceutics. 2022; 14(6):1140. Doi: 10.3390/pharmaceutics14061140
25. Osawa Y, Banno Y, Nagaki M, Brenner DA, Naiki T, Nozawa Y, Nakashima S, Moriwaki H. TNF-alpha-induced sphingosine 1-phosphate inhibits apoptosis through a phosphatidylinositol 3-kinase/Akt pathway in human hepatocytes. J Immunol. 2001; 167(1):173-180. Doi: 10.4049/jimmunol.167.1.173
26. Bosani M, Ardizzone S, Porro GB. Biologic targeting in the treatment of inflammatory bowel diseases. Biologics. 2009;3:77-97. Retraction in: Biologics. 2014; 8:39. Doi: 10.2147/BTT.S60301
27. Neyrinck AM, Van Hée VF, Bindels LB, De Backer F, Cani PD, Delzenne NM. Polyphenol-rich extract of pomegranate

28. peel alleviates tissue inflammation and hypercholesterolaemia in high-fat diet-induced obese mice: Potential implication of the gut microbiota. Br J Nutr. 2013; 109(5):802–809. Doi: 10.1017/s0007114512002206
29. Mcalindon ME, Hawkey CJ, Mahida YR. Expression of interleukin 1 and interleukin 1 converting enzyme by intestinal macrophages in health and inflammatory bowel disease. Gut. 1998; 42(2):214-219. Doi: 10.1136/gut.42.2.214
30. Boakye YD, Groyer L, Heiss EH. An increased autophagic flux contributes to the anti-inflammatory potential of urolithin A in macrophages. Biochim Biophys Acta Gen Subj. 2018; 1862(1):61–70. Doi: 10.1016/j.bbagen.2017.10.006
31. Garcia-Muñoz C, Vaillant F. Metabolic fate of ellagitannins: implications for health, and research perspectives for innovative functional foods. Crit Rev Food Sci Nutr. 2014; 54(12):1584–1598. Doi: 10.1080/10408398.2011.644643
32. Ge G, Bai J, Wang Q, Liang X, Tao H, Chen H, Wei M, Niu J, Yang H, Xu Y, Hao Y, Xue Y, Geng D. Punicalagin ameliorates collagen-induced arthritis by downregulating M1 macrophage and pyroptosis via NF-κB signaling pathway. Sci China Life Sci. 2022; 65(3):588–603. Doi: 10.1007/s11427-020-1939-1
33. Duda-Chodak A, Tarko T, Satora P, Sroka P. Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. Eur J Nutr. 2015; 54(3):325-341. Doi: 10.1007/s00394-015-0852-y
34. Rennick DM, Fort MM. Lessons from genetically engineered animal models. XII. IL-10-deficient (IL-$10^{-/-}$) mice and intestinal inflammation. Am J Physiol Gastrointest Liver Physiol. 2000; 278(6):829-833. Doi: 10.1152/ajpgi.2000.278.6.g829
35. Glocker EO, Kotlarz D, Boztug K, Gertz EM, Schäffer AA, Noyan F, Perro M, Diestelhorst J, Allroth A, Murugan D, Hätscher N, Pfeifer D, Sykora KW, Sauer M, Kreipe H, Lacher M, Nustede R, Woellner C, Baumann U, Salzer U, Koletzko S, Shah N, Segal AW, Sauerbrey A, Buderus S, Snapper SB, Grimbacher B, Klein C. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med. 2009; 361(21):2033-2045. Doi: 10.1056/nejmoa0907206
36. Azizi G, Pouyani MR, Abolhassani H, Sharifi L, Dizaji MZ, Mohammadi J, Mirshafiey A, Aghamohammadi A. Cellular and molecular mechanisms of immune dysregulation and autoimmunity. Cell Immunol. 2016; 310:14-26. Doi: 10.1016/j.cellimm.2016.08.012