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The Effect of *Myrmecodia pendans* Ethanol Extract on Myeloperoxidase Level and Histopathological appearance in Dextran Sodium Sulfate-Induced Colitis Rat

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ABSTRACT

Myrmecodia pendans is a traditional medicinal plant in Merauke district in Indonesia. This study is aimed at examining the effect of *Myrmecodia pendans* ethanol extract (MPE) on myeloperoxidase (MPO) and histopathological appearance of dextran sodium sulfate (DSS) on the colon of rats. The MPE extract was obtained from maceration. All animals were acclimatized with normal feed, and were subsequently divided into 4 groups. Group I (N): normal feed; group II (CC): Carboxy Methyl Cellulose (CMC 1%); group III (MPE100): MPE 100 mg/kg bw; and group IV (MPE200): 200 mg/kg bw. The animals in groups II, III, and IV were induced with DSS for 4 days to obtain colitis model in rats before commencingtreatment for 7 days. MPO levels did not show a significant difference between the treatment groups (p>0.05). MPO levels in treatment groups III (10.14±3.69 ng/mL) and IV (11.54±4.25 ng/mL) were slightly lower than that of group II (14.79±2.73 ng/mL), but were still higher compared to that of group I (9.83±4.04). There was a significant difference in epithelial surface damage and inflammatory cell infiltration between groups I and II (p<0.01). There was also a significant difference in inflammatory cell infiltration between treatment group IV and control group II (p<0.05). It can be concluded that *Myrmecodia pendans* ethanol extract plays a role in reducing MPO levels and decreasing colonic epithelium damage as well as inflammatory cell infiltration.

Keywords: Colitis, Colon histopathology, Myeloperoxidase, *Myrmecodia pendans* extract.

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are inflammatory bowel diseases (IBD).[1] In CD, all segments of the gastrointestinal tract can be affected, mostly to the terminal ileum and colon, whereas in UC disorder confined to the large intestine or colon.[2] The highest prevalence of CD and UC is in Northern Europe, North America, and England.[3] In Australia, from 2011 to 2013 the incidence of UC was around 10 per 100,000.[4] Incidence is increasing in Asia, Africa, Europe, South America, and most developing countries.[3] In Asia-Pacific, Crohn's and Colitis Epidemiology Study (ACCESS), from 2011 to 2013, in 8 Asian countries, the incidence of IBD ranged from 0.5 to 3 per 100,000, and UC around 1 per 100,000.[4] In Indonesia, there is still no epidemiological study on IBD, and the data are still based on hospital records. From the hospital survey conducted from January 2010 to December 2014, there were 176 UC patients. The prevalence of UC was 8.2%, with a frequency of 36 patients in 2010, 27 patients in 2011, 36 patients in 2012, 37 patients in 2013, and 41 patients in 2014.[5]

Plants are widely used as medicine because their properties have been proved to cure various diseases, with relatively minimal side effects if given with appropriate dosage, time of use, and accuracy. The World Health Organization (WHO) argues that plants are a potential source for the discovery of new drugs.[6]

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One of the plants that has the potential and interest of the public as traditional medicine is *Myrmecodia pendans*.[7]This plant has the potential to treat various cancers, tumours, gout, coronary heart disease, haemorrhoids, tuberculosis, migraines, rheumatism, and leukemia.[8] Previous studies have revealed that flavonoids are the main active substances found in *M. pendans*. Flavonoids can be obtained from different extractions, including fractionation of n-hexane. A phytochemical study on *M. pendans* reported active phenolic compounds found in 70% ethanol extract, and boiling water extract, including flavonoids, tannins, phenolics, and alkaloids.[9]

Acute or chronic intestinal inflammation can be modelled by modifying the frequency and the concentration of Dextran Sodium Sulfate (DSS) to be administered.[10] Colitis severity is observed on daily weight measurement, blood in stool, bowel length, and myeloperoxidase (MPO) activity.[11] MPO is an enzyme that is released through neutrophils activity constituting strong prooxidative and pro-inflammatory, measured by enzyme linked immunosorbent assay. The present study was conducted to investigated the effect of *M. pendans* extract on MPO and histopathological appearance of colitis rats that were induced by DSS.

Materials and Methods

Plant material

M. pendans is a plant originated fram Papua island, Indonesia. The plant collection was conducted in January 2020.Voucher specimens were identified and deposited in the herbarium of Medannese, Department of Biology, Faculty of Science, Universitas Sumatera Utara (No.5388/MEDA/2020).

Preparation of *M. pendans* ethanol extract

The 70% ethanol extract of *M. pendans* preparation and the phytochemical test were performed at laboratory in Faculty of Pharmacy of Universitas Sumatera Utara. The dried sliced tubers of

M. pendans were ground using a blender into coarse powder. As much as 250 g of the powdered plant material was macerated in 70% ethanol 2.5 L for 5 days. It was filtered with filter paper ®Whatman. The filtered ethanol obtained was concentrated using a rotary evaporator to obtain *M. pendans* ethanol extract (MPE), continued with drying in an oven at 50°C for 24 hours.[12] The MPE was eventually screened for phytochemicals to trace the presence of alkaloids, flavonoids, glycosides,[13] saponins, tannins,[14] and triterpenes/steroids.[15]

Preparation of animals

The animals were handled in accordance to the Declaration of Helsinki regulations and the ethical rules of conducting experimental research involving animals.[16]Ethical clearance for this study was obtained from the Universitas Sumatera Utara No. 00684/KEPH-FMIPA/2020. A total of 24 male Wistar rats aged 2-3 months with a bodyweight of 200-250 g were used for the study. The rats were divided into 4 groups of 6 animals each following, one week of acclimatization to the new environment. The rats had access to food and water ad libitum**.**

I. (N): Normal; II. (CC): DSS 1% + CMC 1% served as negative control; III.(MPE100): DSS 1% + MPE 100 mg/kgbw and IV. (MPE200): DSS 1% + MPE 200 mg/kgbw.

DSS (®Sigma Aldrich; MW 4000 mv) were administered for 4 days then continued with CMC or extract for 7 days. On day 8, all rats were sacrificed, and their colons were excised for MPO examination and colonic histopathology.

MPO level examination

Colon MPO level examination was conducted using the MPO Elisa Rats Kit (®Bioassay Technology Laboratory). Colon tissue as much as 50 mg was cleaned from the blood with Phosphate Buffer Saline (PBS) Solution pH 7.4. The tissue was homogenized in PBS pH 7.4 with a glass homogenizer over ice, and it subsequently was centrifuged at 2000 - 3000 rpm for about 20 minutes. The supernatant was taken as a sample. The sample and ELISA reagent were put into wells and incubated for 1 hour at 37°C. They were washed 5 times, added solution A and substrate B and incubated for 10 minutes at 37°C. Finally, the stop solution was added to enable reading of the optical density value in 10 minutes.

Histopathological examination

At the end of the treatment, the rats were anesthetized with ketamine xylazine at a dose of 75-100 mg/kg intraperitoneally for 10-30 minutes, then a laparotomy was performed to remove their colons. Samples were fixed in 10% formalin for 24 hours. Gradual dehydration of the samples using 70% alcohol to absolute alcohol was conducted. The samples were cleaned with xylol, infiltrated, and embedded in 56-58ºC paraffin, then stained with hematoxylin and eosin. Histopathological readings of epithelial surface damage and inflammatory cell infiltration used a light microscope with a magnification of 40 and 200.[17] Colon damage was calculated based on the score of surface epithelial damage and inflammatory cell infiltration, which were divided as follows:

A.Epithelial surface damage: 0(No erosion); 1(Erosion <½ mucosal thickness + damage area <25%); 2(Erosion <½ mucosal thickness + area damage <50%); 3(Erosion> ½ thickness of mucosa + area of damage <50%); 4(Erosion> ½ thickness of mucosa + area of damage> 50%); B.Inflammatory cell infiltration: 0(Inflammation of mucosa only); 1(Inflammation of mucosa and submucosa); 2(Inflammation of the transmural with distant spread of inflammatory cells); 3(Inflammation of the transmural with the spread of inflammatory cells is still infiltrated by one inflammatory cell); 4(Inflammation to the transmural with a narrow distribution of inflammatory cells).

Determination of the degree of colonic tissue damage refers to the sum of the scores from the assessment of epithelial surface damage and inflammatory cell infiltration divided by 2. Epithelial damage was catagorized as mild (<1.0), moderate (≥1.0- <2.5), and severe (≥2.5).

Statistical analysis

Data were analyzed with IBM SPSS Statistic 22 software. The statistical test for MPO data was performed using ANOVA, and histopathological data were tested with Kruskal Wallis.

Results and Discussion

Phytochemical screening of M. pendans extracts

The yield from 1.3 kg of powder plant material consisted of 10 g of *M.pendans* ethanol extract (MPE). The MPE contained flavonoids, glucosides, saponins, tannins, and triterpenes/steroids. However, no alkaloids were traced in this extract.

The effect of MPE on MPO levels

MPO levels in the colon of rats in all treatment groups are presented in Table 1. The results showed that MPO level ini MPE- (MPE100 (10.14 ± 3.69ng/ml) and MPE200 (11.54 ± 4.25 ng/ml)) were higher than CC-treated group (14.79 ± 2.73ng/ml). However, statistically, there is no significant different among groups (p>0.05).

The effect of MPE on damage score of the colonic epithelium

Histopathological examination was performed by looking at the epithelial surface damage in the rat colon. The result of damage to the epithelial surface are shown in Table 2.

The results demonstrated that the damage of epithelium in both extract, MPE100 and MPE200, were lower (1.00 ± 0.00 respectively) than CC (1.50 ± 0.54). These results represented that the degree of colonic damage in normal was mild, while in CC-, MPE100, and MPE200-treated groups were moderate. Statistically, the significant different were found only between Normal and CC group (p<0.01).

The effect of MPE on inflammatory cell infiltration score

As shown in Table 3, the extracts decreased inflammatory cell infiltration score. The score in MPE-(MPE100 (1.33 ± 0.51) and MPE200(1.00±0.63)) as well as Normal (0.16±0.40) were lower than in CC-treated group (2.16 ± 0.75). Interestingly, the different were statistically significant found between CC and MPE200 (p<0.01). In the present study, we found that in Normal-treated group, there were damage and inflammation too. This conditions are reasonable because of food-derived antigents.[17,18] Studies have indicated a decrease in MPO levels after receiving MPE100 mg/kgbw and 200 mg/kg bw, but there was no significant difference compared to the group receiving 1% CMC. These results suggested that *M. pendans* has the potency as an anti-inflammatory in DSS-induced colitis rat. As a higher dose showed an increasing effect, we suggested observing using a higher dose than 200 mg/kgbw to provide a higher anti-inflammatory effect.

Table 1: The effect of *MPE* on MPO levels

Groups		MPO level Mean ± SD (ng/mL)
I	Normal	9.83 ± 4.04
II	CC	14.79 ± 2.73
III	MPE100	10.14 ± 3.69
IV	MPE200	11.54 ± 4.25

Table 2: The effect of *MPE* on damage score of colonic epithelium

Groups		Epithelial surface damage score (Mean ± SD)
I	Normal	0.33± 0.51[x]
II	CC	1.50± 0.54
III	MPE100	1.00± 0.00
IV	MPE200	1.00± 0.00

Note: [x]:Group I and Group II (p<0.01)

As the phytochemical screening of this plant resulted in flavonoid compounds in MPE might be playing a role in its biological activity. Research has found that patients with colitis experience change in colonic mucus and a decrease in the effectiveness of the barrier function.[21]DSS causes inflammation of the monolayer of the intestinal epithelium, directs the entry of luminal bacteria and antigens into the mucosa, and allows pro-inflammatory gut into the tissues.[19,20]
Inflammation of the tissues causes the release of inflammatory mediators, one of which is TNF-α. Pre-clinical with pre and post-treatments and control group design was conducted to reveal the effects of *M.pendans* extracts on blood levels of TNF- as a proinflammatory cytokine. *M.pendans* extract decreased TNF-α in pulpitis rats on day 4.[7] Thus, Nucky et al., showed that the ethanol extract of *M. pendans* had an anti-inflammatory effect by decreasing levels of the inflammatory mediator TNF-*α* in the blood in which phytochemical compounds from the fractioned extract were flavonoids.[22]
As shown in Figure 1A, the black arrow indicates that part of the colon that has epithelial erosions that is seen at 40x magnification. Normal: There were no erosions in the colonic epithelium; CC: There were erosions> ½ of the thickness of the mucosa + area of damage <50%; MPE100 and MPE200: Erosion <½ of mucosal thickness + area of damage <25%. Thus, in Figure1B, the black arrow indicates the part of the colon that is infiltrated with inflammatory cells at 200x magnification. Normal: Inflammation is only limited to the mucosa; CC: inflammation in the mucosa, submucosa to transmural (external muscularis); MPE100 and MPE200: inflammation in the mucosa, submucosa to transmural (external muscularis) with a decrease in the number of inflammatory cells.These results suggested that *M.pendans* has potency as an anti-inflammatory in DSS-induced colitis rats, as a higher dose showed an increasing effect.

Table 3: The effect of *MPE* on inflammatory cell infiltration score

Groups		Inflammatory cell infiltration score (Mean ± SD)
I	Normal	0.16 ± 0.40^{x}
II	CC	2.16 ± 0.75
III	MPE100	1.33 ± 0.51
IV	MPE200	1.00 ± 0.63^{y}

Note: ^{x}: Group I and Group II ($p<0.01$); ^{y}: Group II and Group IV ($p<0.05$)

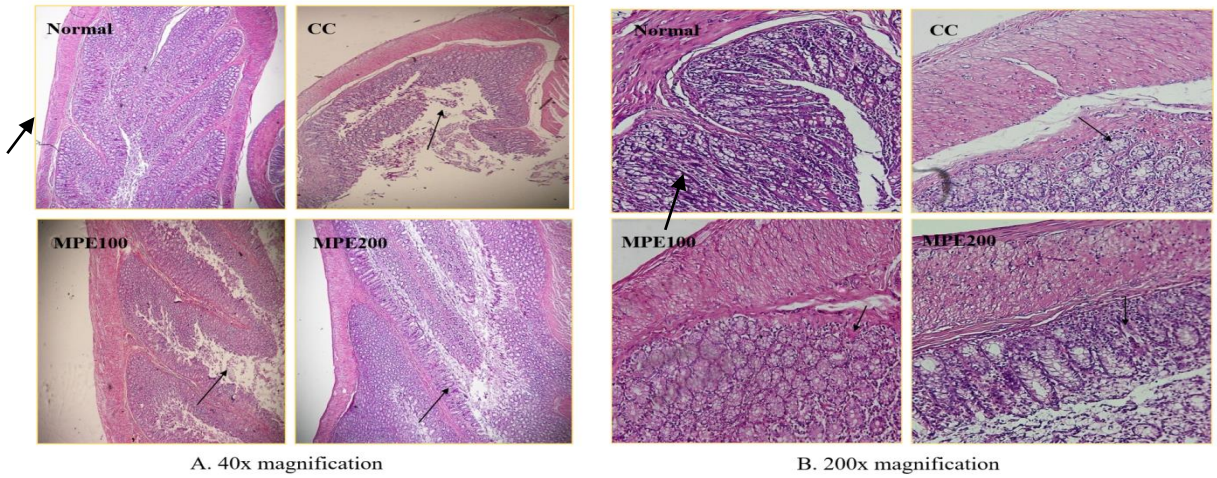

Figure 1: The effect of MPE on histopathology of colon
(A. 40x magnification; B. 200x magnification)

Conclusion

Myrmecodia pendans ethanol extract has potential as anti-inflammatory in colitis rats due to its role in decreasing myeloperoxidase level, colonic epithelium damage and inflammatory cell infiltration score.

Conflict of Interest

The authors declare no conflict of interest.

Authors' 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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