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POTENTIAL OF ETHANOL EXTRACT OF MAHKOTA DEWA LEAVES (*PHALERIA MACROCARPA* (SCHECFF.) BOERL.) TO INHIBIT INFLAMMATION IN MOUSE DISTAL COLON INDUCED BY DEXTRAN SODIUM SULFATE (DSS) AND AZOXYMETHANE (AOM)

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ABSTRACT

Objective: To demonstrates the ability of P. macrocarpa leaf extract to reduce inflammation of the distal colon in DSS/AOM-induced mice.

Methods: *In vivo* experimental research using Balb/c mice induced by 0.2 ml azoxymethane (AOM) 0.1% once and 1% dextran sodium sulphate (DSS) for one week; additionally, ethanol extract of P. macrocarpa leaves, 25 mg and 50 mg, and 0.84 mg acetosal were given orally. The mice were sacrificed after 20 w. Histopathological examination (hematoxylin-eosin staining) was conducted by counting the average number of goblet cells per crypt, inflammatory focus and angiogenesis.

Results: Ethanol extract of P. macrocarpa leaves was able to prevent the decrease in the number of goblet cells ($p<0.05$). However, the administration of ethanol *P. macrocarpa* leaf extract could not reduce focal inflammation and angiogenesis in inflammation of the distal colon.

Conclusion: Ethanol extract of the Mahkota Dewa leaves is able to prevent inflammation of the distal colon by preventing the decrease in the number of goblet cells.

Keywords: Angiogenesis, Distal colon, Ethanol extract, Goblet cell, *Phaleria macrocarpa* leaf

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INTRODUCTION

UC is chronic gastrointestinal inflammation that occurs in the epithelial lining of the large intestine, which causes stomach pain, diarrhea and gastrointestinal bleeding [1]. The global UC incidence is 1.2–20.3 per 100,000 people annually, with a prevalence of 7.6 to 246 per 100,000 people. The incidence and prevalence of Inflammatory Bowel Disease (IBD) is high in European and American populations, whereas in Asian populations, the number of reported cases is low [2]. Patients with UC have a 50% chance of recurrence and 20–30% require colectomy for treatment [3].

UC is a disease caused by immunological mechanisms that occur in people with a genetic predisposition due to an immune system that cannot normally respond to intraluminal antigens; antigens that cause this immunological mechanism are commensal bacteria in the digestive tract [4]. Histopathological examination shows a change in cryptic architecture (shortening or branching crypts), lymphoplasmasitosis basalis and Paneth cell metaplasia [5].

Currently, the choice of UC management depends on the degree of the disease, distribution, causes, frequency of recurrence, extraintestinal manifestations, previous treatment and side effects of the drugs prescribed. The goal of UC management is to provide an improvement in conditions with minimal consumption of steroids while preventing disease complications. The first-line drug given for mild-to-moderate UC is 5-ASA; this drug has been studied by many researchers and shows positive effects in dealing with UC. Unfortunately, 5-ASA can cause a number of side effects, such as diarrhea, nausea, vomiting, headaches and fever, so special attention is required in the use of 5-ASA [6, 7].

Considering these serious health problems, there needs to be an effective, efficient treatment with minimal side effects. Indonesian people have long used many natural substances as medicines; one of these is the Mahkota Dewa (*Phaleria macrocarpa*) leaf. Numerous studies have shown the pharmacological effects of the extract of the P. macrocarpa, such as anti-cancer, antioxidant, antibacterial and anti-inflammatory effects. One of the active ingredients of the P. macrocarpa extract is phalerin, which works by suppressing the expression of COX2 which causes decreased synthesis of prostaglandin so that the inflammatory reaction is reduced [8].

MATERIALS AND METHODS

Sample size

The number of samples used in this study was calculated using the Federer formula (t-1) (n-1) ≥ 15. The number of groups in this study was 5 groups, so the number of replications in each group was:

- (5-1) (n-1) ≥ 15 heads
- (n-1)/4 15/4
- (n-1) ≥ 3175
- n ≥ 4.75

Based on the calculation of the formula above, five mice were obtained for each group; in total, 25 Balb/c mice, which were randomly divided into five groups, were used.

Material

Dextran sodium sulfate (DSS) with a molecular weight of 36,000–50,000 was obtained from Regent Science Industry Limited (RSC), Hong Kong. The ethanol extract of P. macrocarpa leaves was obtained from the Biopharmaca IPB Study Center, Bogor, Indonesia.

Experiment design

In vivo experimental research using Balb/c mice divided into five groups. Each group consists of 5 mice:

1. The normal group (N) consisted of mice that were not given any treatment.

2. The negative group (K-) consisted of mice given 0.2 ml AOM 0.1% w/v ip once for seven days and then given a 1% w/v DSS solution through drinking water every day for seven days *ad libitum*.

3. Positive group (K+) consisted of mice given 0.2 ml AOM 0.1% w/v ip once for seven days and then given an oral suspension of acetosal 0.4% w/v in the amount of 0.21 ml (equivalent to 0.84 mg of acetosal) and a 1% w/v DSS solution with drinking water every day for seven days *ad libitum.*

4. High dose group (DT) consisted of mice given 0.2 ml AOM 0.1% w/v ip once for seven days and then given an extract of 25% w/v P. macrocarpa orally in the amount of 0.2 ml (equivalent to 50 mg extract) and 1% DSS w/v solution with drinking water every day for seven days *ad libitum.*

5. The low dose group (DR) consisted of mice given 0.2 ml AOM 0.1% w/v ip once for seven days, and then given 12.5% w/v P. macrocarpa leaf extract orally in the amount of 0.2 ml (equivalent to 25 mg extract) and a 1% DS w/v solution with drinking water every day for seven days *ad libitum.*

Mice were sacrificed after 20 w of being given treatment according to their group. Distal colonic tissue was then stained with hematoxylin-eosin for histopathological observation.

Histopathological examination

Histopathological observations were made at the Pathological Anatomy Laboratory FKUI, Jakarta, Indonesia. Observations focused on calculating the number of goblet cells per crypt, the focus of inflammation and angiogenesis in six predetermined fields. Observations were made in a double-blind manner. Observations were made at 400-times magnification and then analyzed using ImageJ software (NHI).

Ethical approval

This study passed the ethical review from the Faculty of Medicine University of Indonesia Health Research Ethics Committee with letter number 0891/UN2. FI/ETIK/2018.

Statistical analysis

Statistical analysis was performed using the One-Way ANOVA test, followed by Tukey's Post Hoc test for goblet cell variables and inflammatory focus. Angiogenesis variables were analyzed using the Kruskal-Wallis test because the data distribution was not normal.

RESULTS

Effect of administration of *P. macrocarpa* leaf extract on the number of goblet cells

Goblet cells play a protective function in the mucosal lining of the colon by secreting mucosa [9–11]. Damage to the mucous layer causes an increase in permeability to bacteria and toxins that can damage epithelial cells and cause systemic inflammation, such as UC [12]. In UC, there is a depletion of goblet cells due to inflammation processes. This is related to a decrease in glycosylation of mucin accompanied by the absence of the MUC2 and MUC3 genes in goblet cells [13]. Goblet cell depletion in UC is also associated with impaired induction of Hath1 and KLF4 differentiation factors during inflammation [14]. The effect of P. macrocarpa extract on observed goblet cell counts with hematoxylin-eosin staining using 400-times magnification is shown in fig. 1.

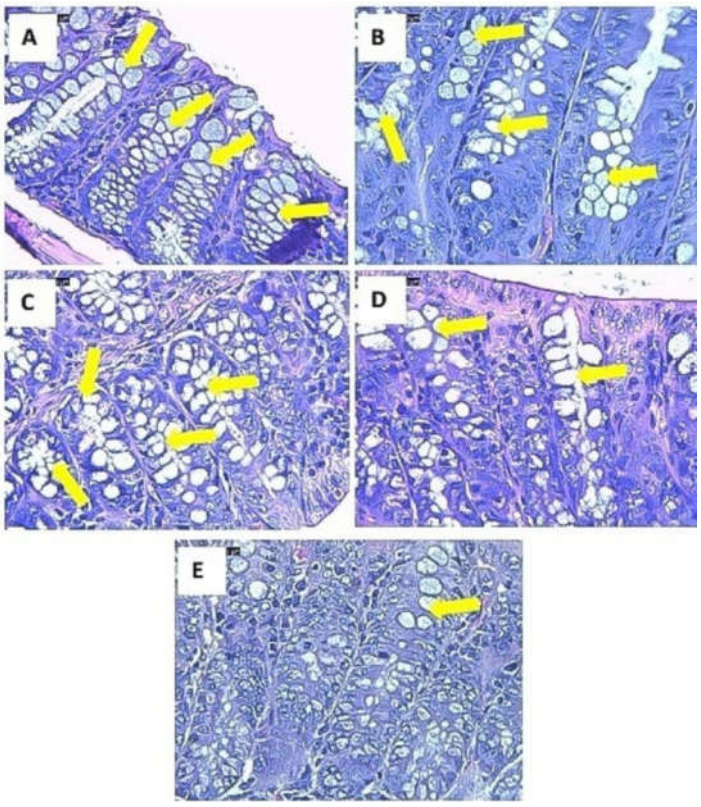

Fig. 1: Effect of administration mahkota dewa extract to the number of goblet cells: (A) Normal group, (B) DSS/AOM+Aspirin 0.86 mg, (C) DSS/AOM+50 mg extract, (D) DSS/AOM+Extract 25 mg and (E) DSS/AOM

The administration of P. macrocarpa leaf extract was able to prevent a significant decrease in the number of goblet cells ($p<0.05$) (fig. 2). The results of this study are supported by a number of other studies. A study by Suprapti *et al.* examined the effect of the P. macrocarpa leaf extract on colon inflammation in mice. From this study, it was concluded that the P. macrocarpa leaf extract can reduce colonic tissue damage and prevent an increase in the expression of COX-2, NOS and β-catenin [15]. A study by Maharani *et al.* showed that the P. macrocarpa leaf extract with a dose of 100 mg, 200 mg, and 300 mg can inhibit a decrease in the number of goblet cells in the colon [16].

Effect of administration of *P. macrocarpa* leaf extract on the amount of angiogenesis

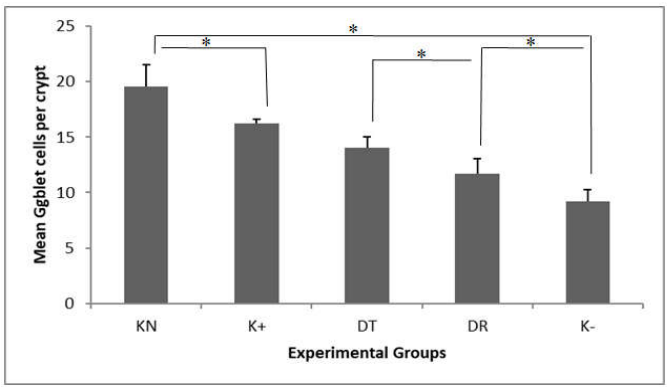

Fig. 2: Administration of Dewa Dewa leaf extract to normal group goblet (KN) cells, (K+) Aspirin 0.86 mg, (DT) DSS/AOM+50 mg extract, (DR) DSS/AOM+25 mg extract and (K-) DSS/AOM

The administration of DSS to the colon was able to increase the number of angiogenesis. In this study, the administration of the *P. macrocarpa* leaf extract did not reduce the amount of angiogenesis in the DSS/AOM-induced distal colitis (p = 0.895).

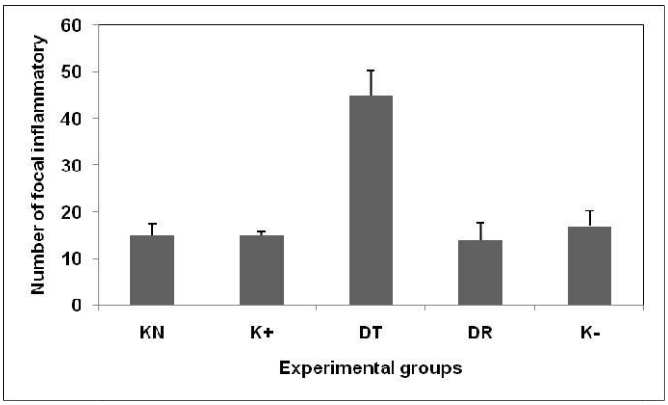

Fig. 3: Effect of administration of dewa dewa leaf extract to the number of angiogenesis (KN) normal group, (K+) Aspirin 0.86 mg, (DT) DSS/AOM+50 mg extract, (DR) DSS/AOM+25 mg extract and (K-) DSS/AOM

Effect of administration of *P. macrocarpa* leaf extract on the amount of inflammatory focus

The focus of active inflammation is defined as cryptic damage due to neutrophil infiltration. The discovery of neutrophil infiltration in crypts and cryptic abscesses indicates an inflammatory focus. Research by Osmond *et al.* showed that the focus of inflammation leads to clinical diagnosis in UC [17, 18]. In UC patients, active inflammation is characterized by the presence of neutrophils in the stool, whereas the severity of the disease is characterized by neutrophil infiltration [19].

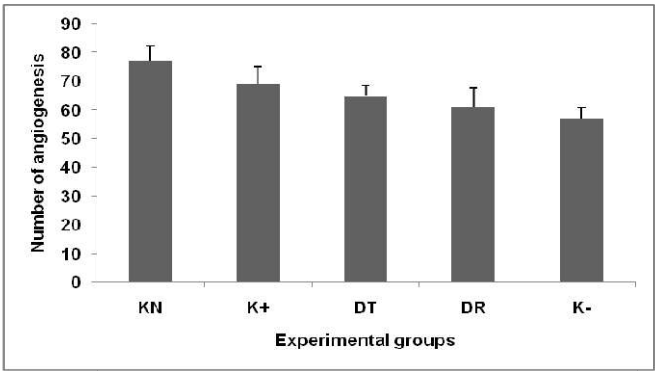

Fig. 4: Effect of administration of dewa dewa leaf extract to the number of focal inflammatory (KN) normal group, (K+) Aspirin 0.86 mg, (DT) DSS/AOM+50 mg extract, (DR) DSS/AOM+25 mg extract and (K-) DSS/AOM

In this study, the administration of *P. macrocarpa* leaf extract did not show a reduction in the amount of inflammation in focus. The amount of inflammatory focus in the control group did not differ significantly with the group given the extract (p = 0.082) (fig. 4).

DISCUSSION

Phaleria macrocarpa is a tropical plant of the Thymelaceae family that commonly grows in Papua, Indonesia. Extracts of *P. macrocarpa* leaves are used for a number of pharmacological activities because of its anti-tumor, anti-inflammatory and anti-fungal effects. Anti-inflammatory activity of *P. macrocarpa* are due to its contents, including tannins, terpenoids, saponins, flavonoids and phenols [8]. The aim of this study is to determine the histopathological effect of distal colon inflammation by inhibition of the ethanol extract of *P. macrocarpa* leaves.

Goblet cells play a protective function in the mucosal lining of the colon by secreting mucosa [9–11]. Damage to the mucous layer causes increased permeability to bacteria and toxins that can damage epithelial cells and cause systemic inflammation, such as UC [12]. In UC, a depletion of the number of goblet cells occurs due to inflammation processes [13]. The results of this study show that there were significant differences in the number of distal colonic goblet cells between the groups given the extract of the *P. macrocarpa* leaves and the group that was only given AOM/DSS ($p<0.05$). Giving 25 mg and 50 mg dosages of *P. macrocarpa* leaf extract can prevent a decrease in the number of goblet cells after the inflammation process occurs; there is a significant difference between the 25 mg and 50 mg doses. In addition, high-dose (50 mg) *P. macrocarpa* leaf extract shows the same anti-inflammation effect (inhibitory decrease in the number of goblet cells) as acetosal.

The results of this study are supported by a number of other studies. The study by Suprapti *et al.* examined the effect of the extract of *P. macrocarpa* leaf on colon inflammation in mice. From these studies, it was concluded that the extract of the *P. macrocarpa* leaf can reduce colonic tissue damage, as well as prevents an increase in COX-2, NOS and β-catenin expression [20]. Studies by Maharani *et al.* showed that 300 mg can inhibit a decrease in the number of goblet cells in the colon [16].

The increased amount of foci of inflammation in UC is caused by the role of neutrophils, which are the first immune cells that respond during the inflammation process. Uncontrolled activation of neutrophils with UC causes damage to the colonic epithelial tissue and allows infiltration into the crypts [21]. Showed the anti-inflammatory effect of *P. macrocarpa* leaf extract by suppressing the amount of inflammatory focus in DSS-induced UC [16].

In this study, the administration of *P. macrocarpa* leaf extract did not show a significant difference ($p>0.05$). The amount of inflammatory focus in the control group did not differ significantly from the group given extract. In addition, the amount of inflammatory focus in the group that was given the highest dose of *P. macrocarpa* leaf extract showed the highest number of inflammatory focus. Differences in results from previous studies may be due to the longer induction period of this trial (20 w), which lead to apoptosis or carcinogenesis.

In UC, continuous ulceration is followed by tissue regeneration. This increases the need for oxygen and nutrient supply to the colon tissue. Under physiological conditions, the process of angiogenesis occurs due to a balance between pro-and anti-angiogenic factors; but with UC, the process of angiogenesis becomes uncontrollable due to chronic inflammation, or what is referred to as 'immune-driven angiogenesis' [22–25]. Administration of DSS does not increase the number of blood vessels in the distal colonic tissue. In addition, administration of acetosal or extracts of the *P. macrocarpa* leaf did not show a decrease in the number of angiogenesis.

The ethanol extract of the *P. macrocarpa* leaves is able to prevent the inflammation process in the distal intestine by preventing a decrease in the number of goblet cells. However, P. macrocarpa leaf extract cannot reduce the amount of angiogenesis or inflammatory focus.

CONCLUSION

The ethanol extract of the Mahkota Dewa leaves (*P. macrocarpa*) is able to prevent the inflammatory process in the distal intestine by preventing a decrease in the number of goblet cells, but it cannot reduce the amount of angiogenesis and inflammatory focus.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no competing interest.

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