THE EFFECT OF MAHKOTA DEWA (PHALERIA MACROCARPA) (SCHEFF.) FRUIT PERICARP EXTRACT ON COX-2 and β-CATENIN EXPRESSION IN MICE COLON INDUCED BY DEXTRAN SODIUM SULFATE
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Abstract
The objective of this research was to investigate the anti-inflammatory effect of mahkota dewa (Phaleria macrocarpa) fruit pericarp extract on COX-2 and β-catenin expression in mice colon induced by dextran sodium sulphate (DSS). The anti-inflammatory activity was performed by DSS-induced colitis model through assessment of COX-2 and β-catenin expression by immunohistochemistry assay at four different doses, i.e. 650 mg/kg, 1250 mg/kg, 2500 mg/kg and 5000 mg/kg. Swiss webster male mice weighing 25-30 g were used for the study. The results of COX-2 optical density score in dose 625 mg/kg, 1250 mg/kg, 2500 mg/kg and 5000 mg/kg were 1.58, 1.52, 1.47, 1.33, respectively. This result was significantly different (p = 0.000) with DSS group that was 2.46. Meanwhile β-catenin optical density score in dose 625 mg/kg, 1250 mg/kg, 2500 mg/kg and 5000 mg/kg were 1.46, 1.37, 1.41, 1.36, respectively. This result was significantly different (p = 0.000) with DSS group that was 2.34. Pericarp extract of P. macrocarpa fruit exhibited anti-inflammatory activity in experimental model shown by suppressing expression of COX-2 and β-catenin.

Keywords: pericarp, Phaleria macrocarpa, Cyxlooxygenase-2, β-catenin, dextran sodium sulphate, colon
Introduction

The risk of colorectal cancer in ulcerative colitis patients was increase with time of exposure to the disease, i.e. 1.6% after 10 years, 8.3% after 20 years, and 18.4% after 30 years. [1] The aetiology of ulcerative colitis is not yet known, but there were several factors that play a role in the development of the disease, among others, genetic disorders that cause T-cell responses become aggressive and excessive to subset of commensal enteric bacteria, environmental factors that can damage the mucosal barrier rapidly and stimulate immune response, and enteric bacterial equilibrium disorders. Excessive immune responses to the colon will cause impairment of epithelial function and epithelial response to pathogens. [2-4]

The current anti-inflammatory therapy aims to control the cardinal signs of inflammation, antagonizing or blocking key pro-inflammatory mediators that are released at the beginning of an acute inflammatory response. However, prolonged use of many anti-inflammatory agents have serious adverse reactions such as gastric intolerance and bone marrow depression. [5] Hence, it is important to search for substances that can promote resolution of inflammation, homeostatic and modulators efficient and which are tolerated by the body. [6] The development of standardized herbal medicines with proven efficacy and safety can be considered as an important source for increasing the access of people toward medicine and offers new therapeutic options. [7] P. macrocarpa known as mahkota dewa is a traditional medicinal plant in Indonesia. Literature indicates in vitro anti-inflammatory activity of P. macrocarpa fruit pericarp extract. [8] Flavonoid content of P. macrocarpa has proven to decrease expression of COX-2, an isoenzyme of the cyclooxygenase enzyme that will form during inflammation and play role in the synthesis of prostaglandin E2 which take part in all the processes of the inflammation signs of such as redness, swelling and pain. [9, 10] Flavonoid also inhibited β-catenin transcriptional activity and decrease the β-catenin accumulation in the nucleus. [11] β-catenin normally bound to E-cadherin as a complex of adhesion, and then degraded by adenomatous polyposis coli (APC), but it can accumulate in the cytoplasm and then move to the nucleus due to mutations. Increased accumulation of β-catenin may be a factor of transcription and increase tumour progression [12,13] This research was undertaken to investigate in vivo anti-inflammatory activity of P. macrocarpa fruit pericarp extract.

Methods

The adult male Swiss Webster mice (Veterinary Laboratory of Research and Development Centre, Ministry of Health, Jakarta) weighing 25–30 g were maintained on a 12/12-h light/dark cycle of relative humidity and temperature (25-28°C). Animals were allowed free access to food and water and acclimatized under laboratory conditions before carrying out the experiments. Ethical approval has obtained from The Health Research Ethics Committee, Faculty of Medicine Universitas Indonesia number 24/UN2.FI/ETIK/1/2017. Dextran sodium sulphate (DSS) 2% purchased from Regent Science Industry Ltd, Hongkong is given through drinking water for 7 days and then punctuated with 7 days without administration of DSS. This cycle is then repeated up to 3 cycles. The dosage of the test drug was designed based on previous publication research [14, 15]. Forty two experimental animals were divided into seven groups with six animals in each group. Mice were sacrificed at the end of week 7 by dislocation of the neck, subsequently colon tissue put into 10% formalin buffer solution. Then paraffin blocks are made in accordance with standard laboratory procedures.

The immunohistochemical staining were performed according to previous study [16] in brief After deparaffinisation and rehydration, samples were soaked by 3% of hydrogen peroxide to eliminate endogenous peroxide and then with 0.01 M of citrate buffer (pH 6.0) in microwave to returns the structure of the antigen, and then spilled with by 5 % normal serum to block unspecific protein. The next step were incubated the specimens with polyclonal antibodies of COX-2 (1:1000 dilution) and β-catenin (1:300 dilution) purchased from Abeam Inc., Cambridge, MA, followed by appropriate secondary antibody, HRP-conjugated streptavidin and 3,3'-diaminobenzidine (DAB) subsequently. Next counterstained with Harris haematoxylin, dehydrated and mounting.
Reading were taken at 400 times magnification and every 5 representative field of view. The interpretation of results were using image J profiler software that will assess the percentage of colour intensity as high positive, positive, low positive, negative and then Optical Density Score (ODS) calculated [17,18] by using the formula:

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\text{ODS} = \frac{(\% \text{ high positive} \times 4) + (\% \text{ positive} \times 3) + (\% \text{ low positive} \times 2) + (\% \text{ negative} \times 1) + \text{mean} \times 100}{100}
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Statistical analysis
The values were expressed as mean standard deviation. P<0.05 was considered significant, denoted by symbol (*). The data was analysed by one-way analysis of variance followed by Tukey multiple comparison using SPSS 20 software.

Results
Normal group showed weak expression of COX-2 in the cytoplasmic of epithelium cell crypt, in contrast to the strong expression in DSS group. While COX-2 expression in \(P. macrocarpa\) extract doses 625 mg/kg and 1250 mg/kg and 2500 mg/kg, stronger than doses 5000 mg/kg but weaker then DSS group, as illustrated in figure 1.

The cytoplasmic \(\beta\)-catenin expression of the epithelial cell crypt in normal group appears weak in contrast to the strong expression in the DSS group. The \(\beta\)-catenin expression in the aspirin group was seen to be stronger than the \(\beta\)-catenin expression in all group of \(P. macrocarpa\) pericarp extract.

Discussion
Previous studies showed that of \(P. macrocarpa\) pericarp contain flavonoid, which inhibit 63.4% NO production and classified as potentially moderate anti-inflammation. [8,20] Flavonoid apparently working through several mechanisms include antioxidative effect, direct free radicals scavenger, immobilization leucocyte, and interaction with enzyme system. Flavonoid of \(P. macrocarpa\) pericarp extract containing kaemferol, myricetin grouped as flavone which working as antioxidant through reaction with free radicals producing more stable and less reactive compound [21]

\(P. macrocarpa\) pericarp extract doses 625, 1250, 2500 and 5000 mg/kg can decrease COX-2 and \(\beta\)-catenin expression on colon tissue significantly different vs DSS group. This is probably role of flavonoid of pericarp extract such kemferol, rutin, naringin and miricetin. Lee et al [22] reported that kaemferol can suppress UVB-induced COX-2 expression. The emphasis of COX-2 expression by rutin is also reported in the research of Choi et al. [23] and Wen-Jing et al. [24] reported that naringin can decrease COX-2 expression in human cervical cancer cell line and Lee et al [25] reported that mirisetin may decrease COX-2 expression through blockade To NF-kB binding activity assessed using electrophoresis mobility test.

\(Phaleria macrocarpa\) pericarp extract groups can decrease \(\beta\)-catenin expression better then aspirin group, caused by flavonoid content of pericarp extract which not only inhibits activation of NFKB and also directly to \(\beta\)-catenin, in addition kaemferol interferes with the binding of the \(\beta\)-catenin complex with specific DNA binding sides to decrease the accumulation of \(\beta\)-catenin in the nucleus. [26] Li et al [27] also proves that naringin can inactivate pathways B-catenin signal on cell line.

Conclusion
All doses of \(P. macrocarpa\) pericarp extract can decrease expression of COX-2 and \(\beta\)-catenin on mice colon epithelial crypt induced by DSS compare to DSS group (p=0.000).

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References


Figure 1: COX-2 expression on the mice colon tissue
A: healthy tissue from normal mice, B: COX-2 expression of DSS group, C: expression of aspirin group, D-G: COX-2 expression of *P. macrocarpa* pericarp extract group dose 652 mg/kg, 1250 mg/kg, 2500 mg/kg and 5000 mg/kg. Yellow arrow showing COX-2 expression in the cytoplasm of epithelial cell crypt. IHC 400x

Figure 2: Graph score of COX-2 expression in mice colon tissue, DSS = dextran sodium sulphate group, ASP = aspirin group, EPMD = *P. macrocarpa* pericarp extract group A: healthy tissue from normal mice, B: COX-2 expression of DSS group, C: expression of aspirin group, D-G: COX-2 expression of *P. macrocarpa* pericarp extract group dose 652 mg/kg, 1250 mg/kg, 2500 mg/kg and 5000 mg/kg. Yellow arrow showing COX-2 expression in the cytoplasm of epithelial cell crypt. IHC 400x, *p=0.000 compared to the DSS group
Figure 3: β-catenin expression on the mice colon tissue
A: healthy tissue from normal mice, B β-catenin expression of DSS group, C: β-catenin expression of aspirin group, D-G: β-
catenin expression of *P. macrocarpa* pericarp extract group dose 652 mg/kg, 1250 mg/kg, 2500 mg/kg and 5000 mg/kg, yellow
arrow showing β-catenin expression in the cytoplasm of epithelial cell crypt. Red arrow showing β-catenin expression in the
nucleus of epithelial cell crypt, IHC 400x

Figure 4: Graph score of β-catenin expression in mice colon tissue, DSS = dextran sodium sulphate group, ASP = aspirin
group, EPMD = *P. macrocarpa* pericarp extract group, * p=0.000 compared to the DSS group