International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.12, No.03, pp 75-84, 2019

The Effects of Ethanol Extracts Temu Mangga (*Curcuma mangga Val.*) to decrease in TNF-α levels, and Colonic Histopathology Picture mice model of *inflammatory bowel disease* (IBD).

Dian Elco Nora[1]*, Kusmardi[2],Anton Bahtiar[1]

[1]Department of Herbal, Faculty of Pharmacy, University of Indonesia, Depok, West Java 16424.
[2]Department of Pathology Anatomy, Faculty of medicine, University of Indonesia, Salemba Raya, Jakarta 10430.

Abstract : Globally it is said that the incidence of *Inflammatory Bowel Disease* (IBD) is 10 cases per 100,000 population. The main focus of the plan is an attempt IBD Therapeutic inhibition of the inflammatory cascade process. Currently the treatment given in cases of *inflammatory boweldisease* is generally still give systemic effects are less profitable and have a promising modality but at a high price. From previous studies Temu mangga (*Curcuma mangga* val) is also known to have anti-inflammatory effects[1]. In this study we want to know effectiveness Temu mangga extract (*Curcuma mangga* Val) in lowering levels of serum TNF-α mice and improvement of colonic histopathology picture mouse model of inflammatory bowel disease (IBD). Measurement of TNF-α with Elisa method and colon histopathology picture by coloring HE.Research housed in Laboratory Animal Health Research and Development Agency, Ministry of Health RI .Laboratorium Phytochemicals Faculty of Pharmacy, University of Indonesia.

In this study, there are six groups of animals . There are K0 treatment (baseline), K PD, K MD4, KMD8, K MD16, DS. And a negative control group for comparison were taken and euthanized from each group that has been induced by DSS at day 9. Statistical calculations using ANOVA parametric test for the decline in TNF-α, and a decrease in goblet cells and Scoring HE (method cooper). The calculations show decreased levels of TNF-α, especially in group K MD16. Where the reduction in TNF-α in K MD16 significantly different with K PD. HE score between KMD4, KMD8, and KMD16 produces results that correspond to dose . The effect of temumangga treatment at a dose of 16 mg / mice provide the best results in cell regeneration compared to a dose of 8 mg / mice and 4 mg / mouse. It is shown on the picture Histopathology of the colon was observed and in scores with cooper method.

Keywords : Inflammatory bowel disease, Temu mangga, TNF-α, Dextran Sodium Sulfate.

DOI: http://dx.doi.org/10.20902/IJPTR.2019.120309

Introduction

Inflammatory bowel disease (IBD) is a condition that describes inflammation of the gastrointestinal tract and chronic idiopathic[2] .Insidens IBD since the end of World War II in Western countries until the early '90s is increasing. Between one and two million people in the United States in particular is expected *Inflammatory Bowel Disease* Ulcerative colitis or Crohn's disease, with the incidence ranging from 70-150 cases per 100,000 people. While in Europe, the incidence of Ulcerative Colitis range 7.3 cases per 100,000 population and the incidence of Crohn's disease about 5.8 cases per 100,000 population[3] .In Indonesia itself there has been no epidemiological studies on IBD, data is still based reports A hospital (hospital based) Simadibrata of Jakarta in 2002 reported 5.2% of cases of Crohn's disease and ulcerative colitis of the total cases colonoscopy performed in Cipto Mangunkusumo Hospital. They said that the incidence of IBD is 10 cases per 100,000 population, 2.2-14,3 ulcerative colitis cases per 100,000 inhabitants and Crohn's disease 3.1-14.6 cases per 100,000 penduduk. Generally, the incidence of ulcerative colitis more than cases of Crohn's disease [4]

Inflammation is a complex reaction that commonly occur in the human body, but the inflammation has repeatedly or continuously will cause damage to DNA resulting in gene mutations, but the body's own immune system to respond and repair the damage. The failure of the immune system in repairing the damage caused by chronic inflammation increases the risk of cancer. It is seen in patients with colorectal cancer, which is preceded by the Inflammatory Bowel Disease (IBD) is a chronic inflammatory disease due to dysregulation of the immune response in the gut [5]

Chronic inflammatory conditions can stimulate the activation of Nuclear Factor-kB (NF-κB) is a transcription factor that plays an important role in the inflammatory process. NF-κB activation will increase the production of proinflammatory cytokines such as IL-1, TNF-α and IFN-γ which, when accompanied by an increasing free radicals can cause DNA damage and trigger carcinogenesis. Thus chronic inflammation that occurs in IBD can lead to colorectal cancer [6,7].IBD is a risk factor for colorectal cancer, IBD are at increased risk of colorectal cancer is 19 times more often than the normal and average 5 % of patients with IBD will develop colorectal cancer in 10-15 years later **[8]**

The main focus of the plan is an attempt IBD Therapeutic inhibition of.cascade prosesinflamasi if not eliminated altogether. In general, the principle of treating inflammation therapy IBD is active IBD quickly to achieve remission; prevent recurrent inflammation by maintaining remission as long as possible; and treat and prevent complications [9].

In previous studies used mice that had been induced by dextran sodium sulfate (DSS) as a model of inflammatory bowel disease.

Numerous studies have shown that inflammation caused by DSS in the colon is reduced by administration of intercellular adhesion molecule-1 (ICAM-1) anti-sense oligonucleotide, Interleukin-10 (IL-10) recombinant, inhibition of 5-lipoxygenase or activity of neutrophils, and neutralization of TumorNecrosis factor-α (TNF-α) [10].

Currently the treatment given in cases of inflammatory bowel disease is generally still give systemic effects are less profitable and have a promising modality but at a high price. Therefore we need an alternative drug discovery from plants that are effective, easy to obtain and secure in both preventing and curing *Inflammatory Bowel Disease* (IBD) .And to add inventory of medicinal plants that are useful as a complementary therapy *Inflammatory Bowel Disease*.Herbal medicine has been developed to complement the role of synthetic drugs that have the possibility of effective, but it still needs to be done to discover and effectiveness of herbal remedies that exist for this. Various compounds that can serve as an anti-inflammatory are contained in medicinal plants. One of the most widely used are Temu mangga (*Curcuma mangga* Val). From previous studies Temu mangga(*Curcuma mangga* val) is also known to have anti-inflammatory effects [1].

Temu mangga also have sitotosik effect against colon cell culture (HCT 116 and HT-29). Which is a model of colon cancer cells **[11].**

Based on the above, researchers interested in conducting research to know whether ethanol extract temu mangga rhizome (*Curcuma mangga* Val) effect on improvement of colonic inflammation in mice induced by

Dextran Sulfate Sodium (DSS).

From previous studies temumangga have antioxidant activity, namely by means of methanol extract and water Curcuma mangga (CMM and CMW) at a dose of 100 πg / ml showed inhibition of lipid peroxidation (LPO) at 78% and 63%, Cyclooxygenase enzymes COX-1 55% and 33 %, and COX-2 of 65% and 55%. At the same concentration, CMM and CMW is able to inhibit human tumor cells 0-46%. [12].

other research results also showed antioxidant activity of ethanol extract of Curcuma Mangga higher than methanol extract and acetone. [13]

compounds in temumangga one of them is curcumin and curcuminoid. In previous studies also note that curcumin / curcuminoid can inhibit inflammatory bowel disease.[14]

The content curcuminoid in retrieval of mango that is equal to 0:18 to 0:47% detected using HPLC method photodiode array detection [15].

Dextran Sodium Sulfate have mechanisms in induction of the colon by affecting the membrane that disrupts the barrier permeability and lead to colitis induced intestinal mucosa.Compounds dextran sulfate sodium (DSS), which is administered orally through drinking water can induce colitis in mice with the same picture on the UC in humans. Results of previous studies show that kolitis only occurs in the large intestine and especially in the distal colon and rectum. At the First time visible inflammatory cell infiltrate consisting of macrophages, neutrophils and eosinophils, followed by kriptitis and crypt abscess and ends erosions and ulcers mucosa with a flared base [16].DSS affect the loss of epithelial barrier function and lumina of the organism or its products into the lamina propria. It stimulates the innate and limphoid element and produce proinflammatory cytokines and chemokines. And also led to an influx of cells with cytotoxic potential such as neutrophils and macrophage inflammatory.[17].Histological changes in DSS-induced colon can be divided into acute and chronic. Type of histological changes in the acute phase of DSS induced colitis attributable characterized by mucin depletion, epithelial degeneration, and nekrosisi indicated disappearance of epithelial cells. Furthermore, there is infiltration of neutrophils in the lamina propria and submucosa, kriptitis, and crypt abscess [18].In this DSS induction due to the effects of cytokines produced led to increased TNF-α and IL-6 is produced by macrophages formed on DSS colitis [17].In ulcerative colitis often decrease food intake, malabsorption, increased loss of nutrients, increase in energy demand, and interactions between drugs with nutrients that causes a colitis sufferer ulseratif having weight loss [19].

Materials and Methods

Experimental Animals

The population is male Swiss Webster mice aged 8-10 weeks with an average weight of 25-30 grams ± obtained from the Laboratory Animal Research and Development Ministry of Health. The sample is a colon of mice populations that have been induced DSS orally ad libitum (via drinking water), a week later the mice were given a solution of ethanol extract of Temu mangga Rhizome (Curcuma Mangga Val) and every day for 14 days.

Chemicals and botanical materials

Materials used in the study include: ethanol 96%, Temu Mangga obtained from Garden Study Center medicinal IPB, dextran sodium sulfate BM 500,000 (Sigma Chemical Company), Sodium carboxy methyl cellulosa / Na CMC, Xylol, Formalin, Xylasin, KetaminAlkohol absolute 90% and 70%, Mouse TNF-α ELISA ready-set-Go

Temu mangga extract preparation

Temu mangga rhizome washed, chopped thin, dried in an oven at a temperature of 40 ° C to dry completely. Simplicia dried mashed up into a powder and then sifted. A total of ± 1 kg simplisia put in maserator, plus 5 L of ethanol (96%) to the overall submerged powder, stirring and steeped for 24 hours, then difiltrasi.Residu plus ethanol (96%) again and again soaked 24 hours. Maceration done 3 times. The filtrate is

collected and dried using a rotary evaporator to obtain a thick extract. To use the extract orally, condensed extract dissolved in a solution of NaCMC 0.5%. How to setup CMC as follows: NaCMC weighed 500 mg, dissolved in 1000 ml of distilled water with the aid of magnetic stirrer

Study design and carcinogen administration

This study must obtain ethical approval from the Ethics Committee of Research and Development of the Ministry of Health (Research and Development Agency).Each group of mice given DSS solution of 2% w / v adlibitum in 100 ml of water. DSS solution was replaced every day and given for 9 days, for groups of 2-6 mice.

In this study, the treatment group number is 6, then the number of replicates in each group is: $n \geq 4$ or $n = 5$ Thus, in this study used 36 male Swiss Webster mice were divided into 6 groups (each group consisting of 6 tail), namely:

group:

1. Mice untreated group called K-0
2. DSS (DSS solution of 2% w / v daily for 9 days) . followed by(0.1 mL suspension of 0,15 mg prednisone equivalent to 60 mg of prednisone in humans given for 14 days) is commonly called the PD group
3. (0.1 mL Temu mangga 4 mg / 20 gram mice orally for 14 days) + (DSS solution of 2% w / v ad libitum each day during the first 9 days / early) called the group MD 4
4. (0.1 mL Temu Mangga 8mg / 20 Gram mice orally for 14 days) + (DSS solution of 2% w / v ad libitum each day during the first 9 days / early) called the group MD 8
5. (0.1 mLlarutan Temu Mangga 16mg / 20 Gram mice orally for 14 days) + (DSS solution of 2% w / v ad libitum each day during the first 9 days / early) group called MD16
6. DSS (DSS solution of 2% w / v daily for 9 days) followed by Na CMC group called DS.

Mice were sacrificed after 9 days post-induction of the DSS and the last day after the appointment extract of temu mangga in the treatment group. euthanized mice done by anesthesia with ketamine 80-100 mg / KgBW and xylazine10 mg / IP to mice die, then dissected and taken their colon.

Histological assessment

Is inserted into the colon of mice formalin10% buffer solution, to do paraffin blocks. Then made preparations with hematoxylin-eosin staining to observe changes their hystopatologi. Stages of making preparations histology includes fixation, dehydration and infiltration, paraffin infiltration, embedding, sectioning, and pasting the object glass . Stain hematoxylin-eosin process begins with the deparafinasi using xylol and then continued with the process of rehydration with absolute alcohol I, II, and III respectively for 5 minutes, alcohol 95%, 90%, 80% and 70% respectively each masig for 5 minutes. Preparations are washed with running water for 15 minutes and continued for 5 minutes.After Aquadest the preparations stained with dye eosin for 5 minutes and the water distilled water for 5 minutes. After the preparations colored, made of dehydrated with alcohol 70%, 80%, 90% and 95% respectively for a few seconds and continued with alcohol 100% I, II and III respectively 2 minutes. Once that is done with the clearing process Xylol I, II and III for 3 minutes and covered with a glass lid. Mixture which has been dyed subsequently observed under a microscope Olympus BX 51. The observations conducted from 40x to 1000x magnification. Observations were made to observe the damage villi and infiltration of inflammatory cells in the lamina propria and goblet cells.

Histological examination of mucosa colon for goblet

Count of goblet cells was quantified by counting the goblet cell number in ten randomly selected visual fields at mucosa colon region under 400 x in colon sections from DSS-treated Swiss Webster mice. All slides were analyzed by double investigator who was blinded to the treatment groups. The comparison of goblet cells in all groups was performed by averaging out the numbers of goblet cells in ten random fields in each colonic section. Ten selected fields shown are representative of all stained sections.

Histological examination with Cooper method

A scoring system to determine the degree of tissue damage according to Cooper et al

Scoring	Morphology criteria
0	Normally
1	*Goblet cells dysplasia/atypical ephitel cells* /inflammation
2	*Goblet cells dysplasia + atypical ephitel cells* /inflammation
3	*Goblet cells dysplasia + atypical ephitel cells* + inflammation

Determination of TNF-α

Tests will be performed with the Mouse TNF alpha ELISA Ready- set- Go, the kit to determine the levels of TNF-α in mice. This kit consists of: Capture antibody, antibody detection, ELISA Powder Coating Buffer, Assay diluents, Detection Enzyme, substrate solution, standard cytokine TNF-α, microplate, as well as wash buffer concentrate solution.

Making the TNF-α standard curve

Standard curves were used to determine the levels of TNF-α in the sample. The standard used is a recombinant cytokines TNF-α. Standard solution is required together with the sample, having acquired absorption standard solution, made curve TNF-α, and known equation of the standard curve.

Statistical analyses

To test the hypothesis of the existence of differences in the control group to test group at each extract performed one-way ANOVA followed by Tukey's test. Before conducting the test determined the data distribution with the Kolmogorov-Smirnov test. All data were analyzed using SPSS version 22.

Results

Effect extract ethanol Temu mangga on body weight:

There was an increase body weight in all groups compared with the beginning of the experiment (Figure 1).

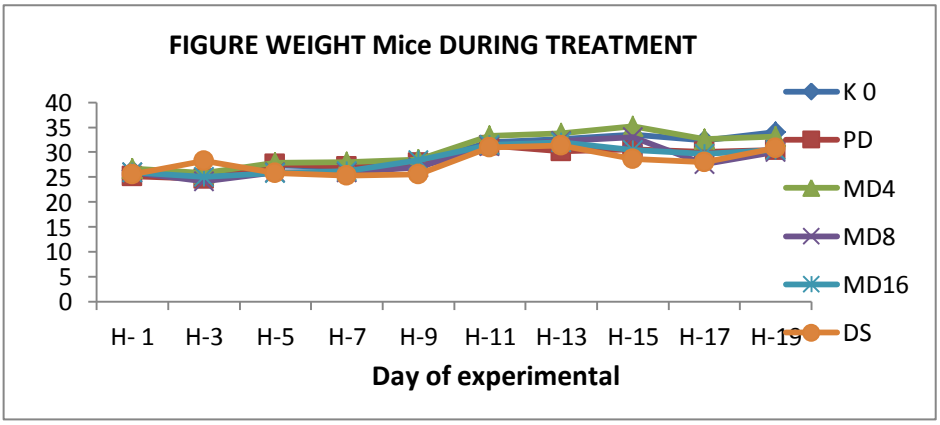


Figure 1.Body weights of mice. Body weights expressed as grams, where the interactive effects of duration of experimental (week) and the group were not significant (p>0,05)using Kruskall-Wallis test.Datas are reported as mean ± standard deviation, n=4/group.

Effect of extract ethanol temu mangga on goblet cells count:

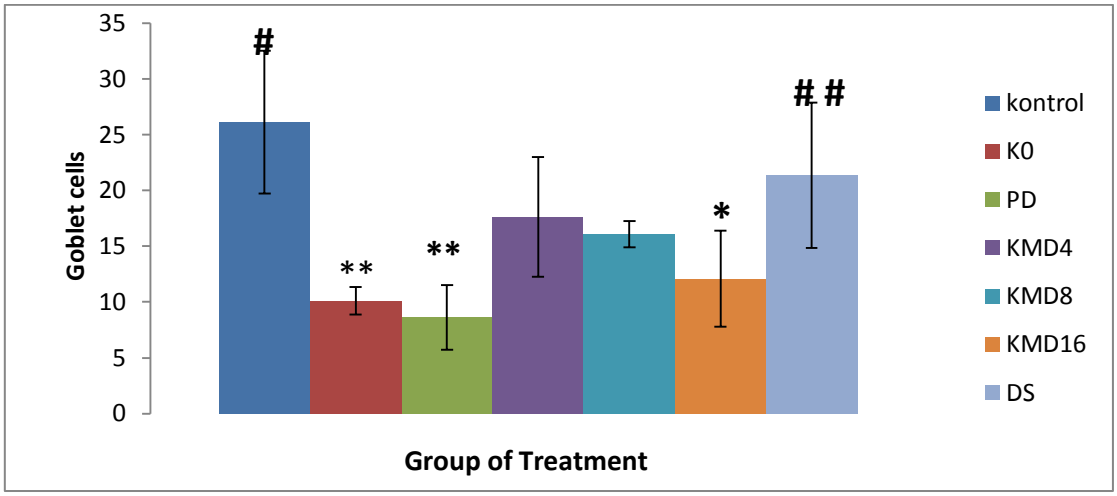


Figure 2. graph of Effect of extract ethanol temu mangga on goblet cells count

From the graph above, it was shown dysplasia The number of goblet cells in the control group differed significantly with K PD. The number of goblet cells at baseline K0 which is normal animals, not significantly different with K PD.the number of DS goblet cells was not significantly different with control.The number of goblet cells in the treatment group KMD4, KMD8 and KMD16, was not significantly different, although showing a decrease appropriate dose leveleffect. The number of goblet cells in KMD16 not significantly different with KPD. From the above results show that the increase of goblet cells in the control group and the group induced euthanized on day 9 showed inflammation of the colon and prednisone showed an improvement in colonic inflammation. Similarly, the provision of Curcuma mangga dose of 4mg, 8mg and 16mg per mice also provide improvement effect against colon inflammation is characterized by a decrease of goblet cells and other inflammatory signs were seen in histology. However, a dose of 16 mg has the highest ability to improve inflammatory doses than others.

Scoring to determine the degree of colon tissue damage

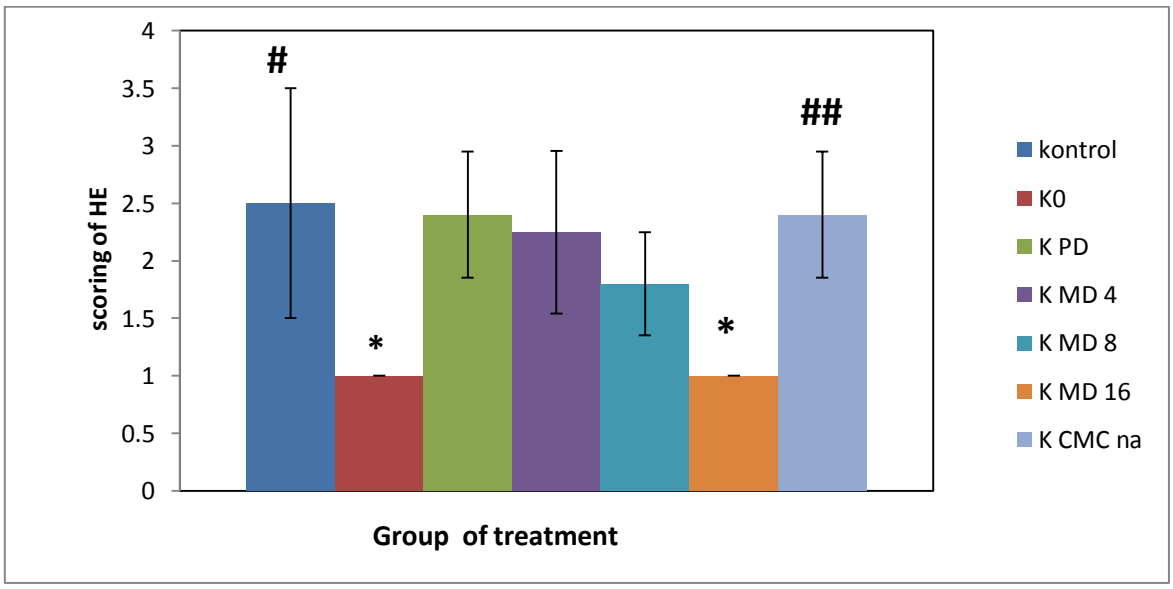


Figure 3. graph of Scoring to determine the degree of colon tissue damage

From the graph above table is obtained, HE scores between the control group (K0) and the group of Na CMC (DS) did not differ between groups bermakna.Skor HE KMD16 and baseline groups were not

significantly different K0, this means that the appointment mango extract 16 mg dose / mice, can regenerate cells from necrosis because of inflammatory state, be like a normal state (baseline).

HE score between KMD4, KMD8, and KMD16 produces results that correspond to dosis.Efect temumangga treatment at a dose of 16 mg / mice best on cell regeneration dose than 8 and 4 mg / mouse.

HE score KMD4 and KMD8 treatment groups did not differ significantly with KPD group, however KMD4 and KMD8 show values lower score. This shows that ekstract of temumangga doses of 4 and 8 mg / mice, candemonstrate the ability to regenerate cells, although not as good as the dose of K MD16.

Determination of serum levels of TNF alpha in animals

Before calculating the levels of TNFα of test samples, first made standard calibration curve TNFα as follows :

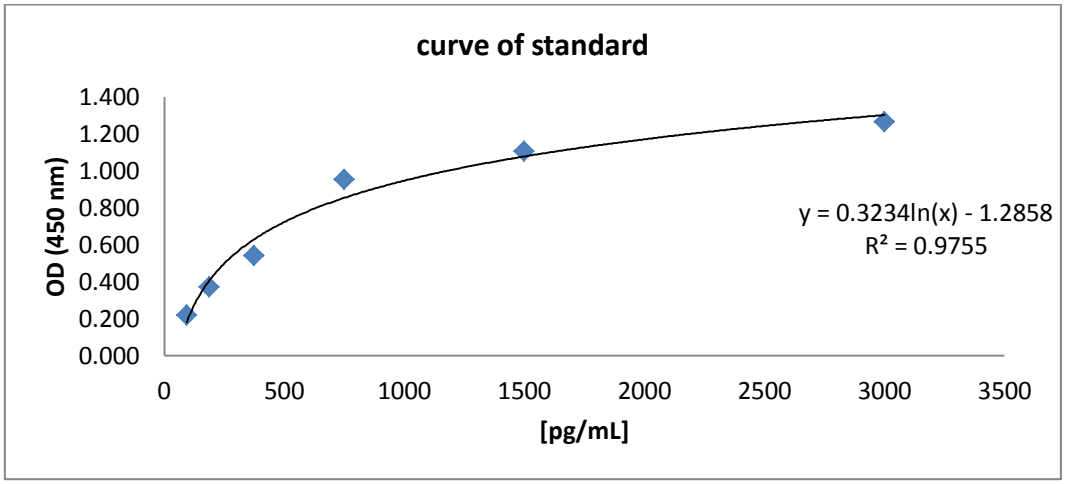


Figure 4. TNF-α calibration curve

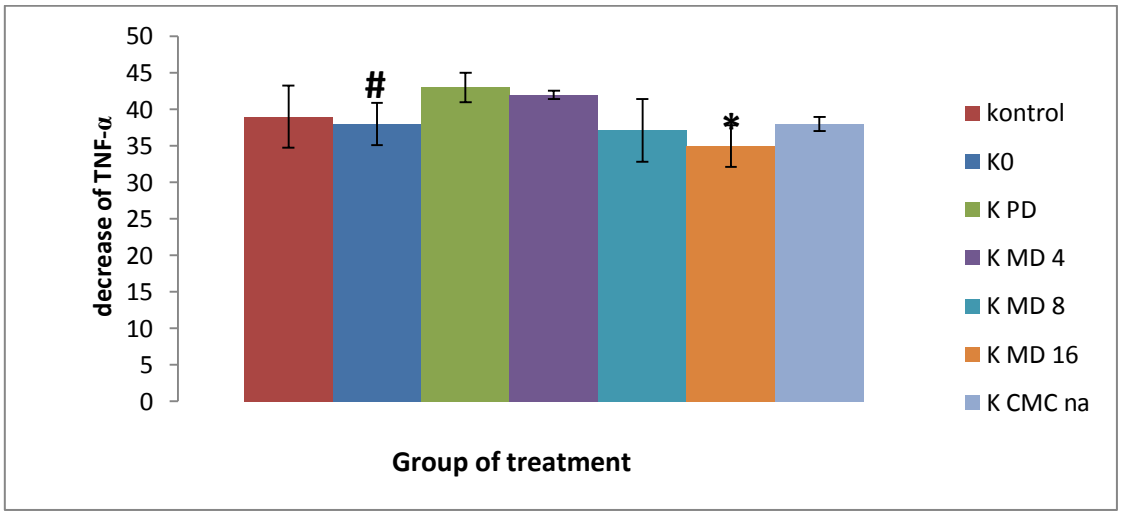
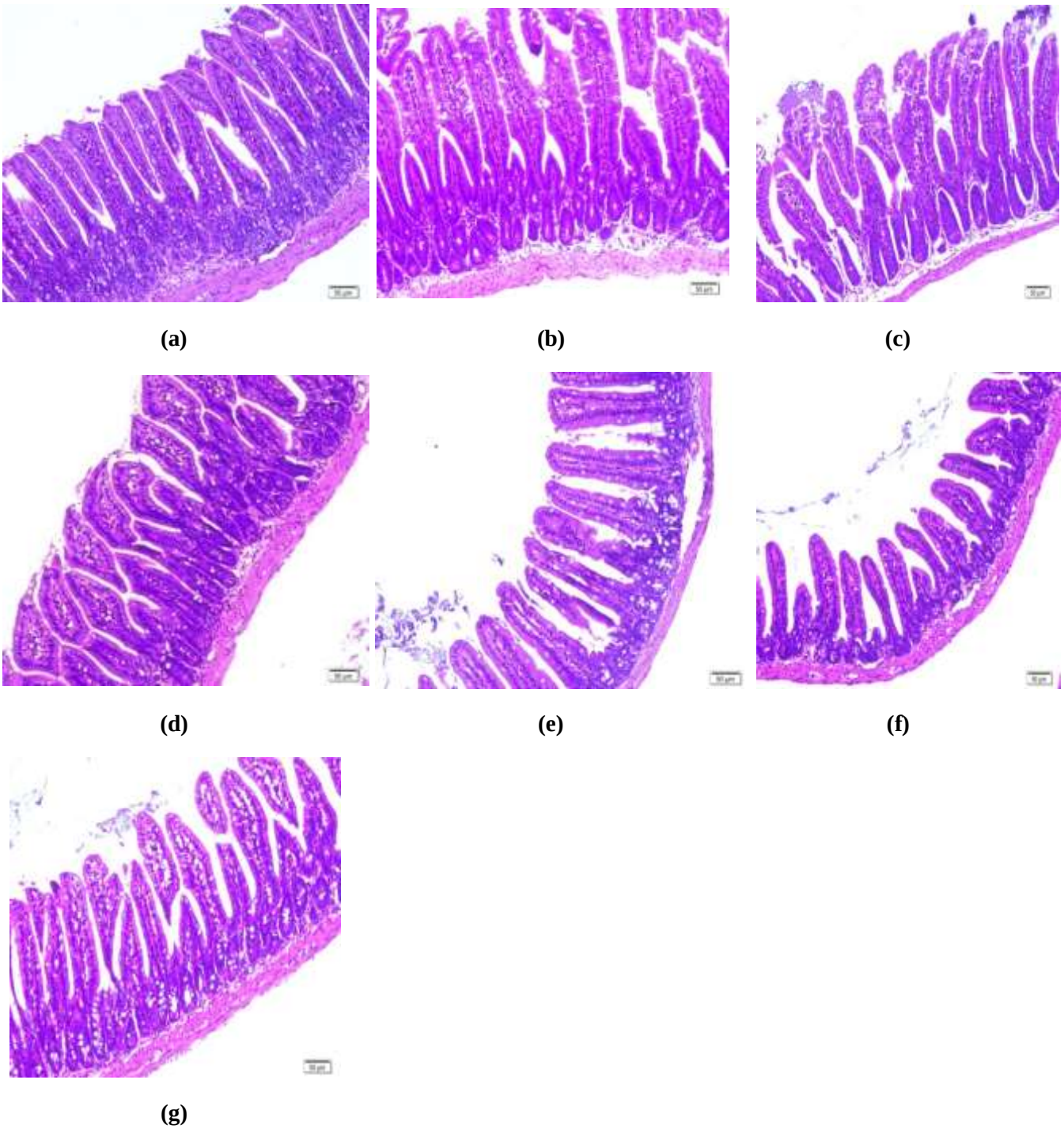


Figure 5. Graph of Determination of serum levels of TNF alpha in animals

Parametric statistical test used to analyze the data TNF-α levels in the serum of experimental animals. Tukey HSD from further tests were done shows reduced levels of TNF-α in KMD16 group showed significantly different results with K PD. KMD 16 also showed a decrease in TNF-α were significantly different with K MD4. Although in the group KMD 16 showed no significant difference with the group other than PD and KMD4 but when compared to other groups then KMD16 showed a decrease in TNF-α are lower when compared to other groups. From the above it can be stated that the decline in TNF-α the lowest occurred in

group K MD16. It is related to the content of the extract of Curcuma mangga ie curcumin and kurkuminoid which has the effect of inhibiting TNF-α as a pro-inflammatory cytokine .



(a) **(b)** **(c)**

(d) **(e)** **(f)**

(g)

Figure 6. Effect of Temu mangga extract on the histological of goblet cells in mice mucosa colon evaluated by hematoxylin and eosin staining 400x. (a) Control(euthanasia after induced DSS 9 days), (b) K0 (baseline), (c) K PD, (d) K MD4, (e) K MD8 , (f) K MD16, (g) DS

Discussion

In general, it is estimated that the process begins IBD pathogenesis of infection, toxins, bacterial products or colonic intraluminal diet in susceptible individuals and is influenced by genetic factors, immune defects, the environment, causing a cascade of inflammation in the intestinal wall. Many inflammatory

mediators have been identified in the pathogenesis of IBD. The cytokines released by macrophages in response to various antigenic stimulus, binds to receptors and produce a variety of effects autocrine, paracrine, and endokrin. Cytokines change into the T cell lymphocytes which T helper cells-1 (Th-1) have a rule of pathogenesis of Crohn's disease and T-cell helper 2 (Th-2) plays a role in Ulcerative Colitis. Imunity response will eventually damage the gastrointestinal mucosa and trigger a cascade of chronic inflammatory processes[20]

The main focus of the plan is an attempt IBD Therapeutic inhibition of the inflammatory cascade process if not eliminated altogether. In general, the principle of therapy is to treat inflammatory active IBD .IBD rapidly to achieve remission; prevent recurrent inflammation by maintaining remission as long as possible; and treat and prevent complications[9]

n previous studies used mice that had been induced by dextran sodium sulfate (DSS) as a model of inflammatory bowel disease.

Numerous studies have shown that inflammation caused by DSS in the colon is reduced by administration of intercellular adhesion molecule-1 (ICAM-1) anti-sense oligonucleotide, Interleukin-10 (IL-10) recombinant, inhibition of 5-lipoxygenase or activity of neutrophils, and neutralization of TumorNecrosis factor-α (TNF-α)[10]

From the results of phytochemical, Temu mangga contains curcuminoid / curcumin. it is known that curcumin / curcuminoid can inhibit *inflammatory bowel disease*[14]

In this study, DSS is used to make animal models of IBD. Compounds dextran sulfate sodium (DSS), which is administered orally through drinking water can induce colitis in mice with the same symptom picture at UC in humans. Results of previous studies show that colitis occurs only in the large intestine and especially in the distal colon and rectum. At first it looks infiltrates of inflammatory cells consisting of macrophages, neutrophils and eosinophils, followed by cryptitis and crypt abscess and ends erosions and ulcers mucosa with a flared base[16]

DSS affect the loss of epithelial barrier function and lumina of the organism or its products into the lamina propria. It stimulates the innate and limphoid element and produce proinflammatory cytokines and chemokines. And also led to an influx of cells with cytotoxic potential such as neutrophils and macrophage inflammatory.[17]

In this DSS induction due to the effects of cytokines produced led to increased TNF-α and IL-6 is produced by macrophages formed on DSS colitis[17]

In this study, mice were induced with DSS 2% for 9 days, followed by treatment dosing Intersection mango 4mg / mice, 8 mg / mice and 16mg / mouse. other than that given prednisone as a control positif.

From the results of this study found that the appointment of temu mangga *(Curcuma mangga* Val) can inhibit inflammation characterized by improvements in picture histopathology at doses of 16 mg / dose mice than the others. At a dose of 4 mg / mice and 8 mg / mice also had improved but not as good a dose of 16 mg / mouse. Neither the number of goblet cells. Temu mangga can reduce the number of goblet cells dysplasia significantly, especially doses of 16 mg / mouse. , In comparison decreased levels of TNF-α is also known dose of 16 mg / mice have a significant ability to lower TNF-α compared with prednisone. This may be because the content curcuminoid, so temu mangga have anti-inflammatory and antioxidant. Temu mangga in addition to inhibit inflammation, also can regenerate damaged cells.

References

1. Ruangsang, P., Tewatrakul, S., dan Raenmongkol, W.2010. Evaluation of the analgesic and antiinflammatory activities of *Curcuma mangga* Val and Zijp rhizomes. Journal of natural medicine. 64:36-41
2. Kuhbacher T, Folsch UR. Practical guidelines for the treatment of inflammatory bowel disease. World J Gastroenterol 2007; 13(8): 1149 – 55.

3. Loftus EV Jr, Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, Zinsmeister AR. Ulcerative colitis in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. Gut 2000; 46(3): 336-43.
4 Kelompok Studi Inflammatory Bowel Disease Indonesia. Konsensus nasional penatalaksanaan infl ammatory bowel disease (IBD) di Indonesia. Jakarta: Perkumpulan Gastroenterologi Indonesia ,2011
5. Tanaka T., et al. 2003. A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate. Cancer Sci. 94:965–73.
6. Kim S. et al. 2009. Carcinoma-produced factors activate myeloid cells trough TLR2 to stimulate metatasis. Nature 457, 102-106
7. Tanaka T., Oyama T., Yasui Y. 2008. Dietary supplements and colorectal cancer. Curr Nuetraceut Res. 6:165-88.
8 Lisiane B. Miera, James M. Bugini, Stephanie L. Green., et al. 2008. DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice. J. clin. Invest. 118:2516-2525.
9. Tamboli CP. Current medical therapy for chronic inflammatory bowel disease. Surg Clin N Am 2007; 87: 697 – 725
10. Diaz-Granados, N., Howe, K., Lu, J., McKay, D.M.. 2000. Dextran SulphateSodium-induced colonic histopathology, but not altered epithelial iontransport, is reduced by inhibition of phosphodiesteraseactivity.http:///www.pubmedcentral.ni.gov/articlerender.fcgi?artid=1850075.
11 Malek SNA, Lee GS, Hong SL, Yaacob H, Wahab HA, Weber JFF, Shah SAA (2011). Phytochemical And Cytotoxic Investigations Of Curcuma
12 (Liu, Y., et al., 2012).
13 Pujimulyani D .2006 . sifat antioksidatif ekstrak kunir putih (*curcumamangga val*) dengan pelarut aseton , etanol, metanol. Biota XI : 14-19
14. Brumatti LV, Marcuzzi A, Tricarico PM, Zamin V, Girardelli M, Bianco AM, 2014. Review curcumin and Inflammatory Bowel Disease: Potensial and Limits of innovative treatment., Molecules 2014,19 .21127-21153., ISSN 1420-3049.
15. Bos R, Windono T, Woerdenbag HJ, Boersma YL, Koulman A, Kayser O., HPLC-photodiode array detection analysis of curcuminoids in Curcuma species indigenous to Indonesia., Phytochem Anal. 2007 Mar;18(2):118-22
16. Stevceva, L., Pavli, P., Husband, A., Ramsay, A., dan Doe, W.F. 2001. DextranSulphate Sodium-induced Colitis is Ameliorated in Interleukin 4 DeficientMice. Genes and Immunity. 2: 309-16
17. Kiesler P, Fuss J I dan Strober W . Experimental Models of Inflammatory Bowel Disease (Review). Cell Mol Gastro-enterol Hepatol 2015;1: 154-170.
18. Martina P, Anton C, (2012). Review article Dextran Sodium Sulphate Colitis Mouse Model: Traps and Tricks. Journal of Biomedicine and Biotechnology. Article ID 718617.
19. Shamir R.. 2009. Nutritional aspects in inflammatory bowel disease http://www.ncbi.nlm.nih.gov/pubmed/19300135.
20. Rowe WA, Katz J. Inflammatory bowel disease 2012. http://www.medscape.com

*****