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THE EFFECT OF CIPLUKAN EXTRACT (Physalis angulata L.) AS ANTIPSORIATIC AND TO LYMPHOCYTES COUNT OF SKIN TISSUE IN PSORIASIS MICE MODEL

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ABSTRACT

Psoriasis is a chronic skin disease in form of papule-shaped lesion and erythematous plaques with thick white scales. The pathogenesis of psoriasis involves IL-23/Th-17 cytokine pathway that contributes the activation of T-lymphocytes and proinflammatory cytokines. Treatment of psoriasis using methotrexate has inhibitory effect of the synthesis of nucleic acid towards T-lymphocytes and keratinocytes. Ciplukan (Physalis angulata L.) has anti-inflammatory potential effect which contains steroid, flavonoid, alkaloid, and physalin that may inhibit lymphocyte activation and proinflammatory cytokine production. The study is used the method of experimental study with post test only with control group design. Thirty five female mices were divided into 7 groups. The parameters of this study is anti-psoriatic (PASI and Baker's score) and lymphocytes count in psoriasis mice model. The results of Kruskal-Wallis, PASI and Baker's score showed that p=0,001 (p<0,05) and the result of lymphocytes count using One Way ANOVA showed that p=0,001 (p<0,05). The 800 mg/kgBW dose of ciplukan extract showed the largest decrease on PASI score and lymphocytes count, and the 1200 mg/kgBW dose one showed the largest decrease on the Baker's score. The present of the 800 mg/kgBW dose of ciplukan extract gives the most optimal effect in reducing PASI score and skin tissue lymphocytes count in psoriasis mice model that were not significantly different with the treatment control group, while 1200 mg/kgBW dose one gives the most optimal effect in reducing Baker's score that were significantly different with the treatment control group.

Keywords: Ciplukan extract, lymphocytes, methotrexate**,** Physalis angulata L

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INTRODUCTION

Psoriasis is a chronic skin condition that is not infectious but can make sufferers disabled and reduce their quality of life (WHO, 2016). The skin in psoriasis patients appears dry and peeling due to the acceleration of keratinocyte proliferation and can spread throughout the body (Keumala *et al*., 2019). Psoriasis is becoming more common every year, and its incidence varies significantly throughout the world. According to data from Prof. Dr. Margono Soekarjo Hospital in Purwoketo, patient with psoriasis visited the hospital 562 times in 2016 and 705 times from January to August 2017 (Astrid *et al*., 2018; WHO, 2016).

The pathogenesis of psoriasis involves the interleukin-23/T helper-17 pathway. The cytokine IL-23 induces the differentiation of T-naive cells into Th17 cells in the dermis, resulting in IL-17 cytokines, which play an essential role in creating inflammatory and chemotactic environments by increasing IL-6, IL-8, and keratinocyte expression of ICAM-1 (Polese *et al*., 2020; Madonna *et* al., 2015; Lowes *et* al., 2014;). In addition, there are Th1 and T cytotoxic 1 (Tc1) lymphocyte cells produced by IL-12 cytokines that can cause lymphocyte infiltration in tissues characterized by keratinocyte hyperproliferation acanthosis and hyperkeratosis of skin tissue (Lorthois *et al*., 2019; Di Cesare *et al*., 2009; Conrad *et al*. 2007).

Methotrexate (MTX) as a psoriasis therapy, especially for moderate to severe degrees, has serious side effects such as myelosuppression, pulmonary fibrosis, and gastrointestinal disorders, even hepatotoxicity (Menting *et al*., 2016; Pathirana *et al*., 2009). Long-term treatment in psoriasis is associated with an increased risk of drug side effects, high costs, and low patient adherence (Keumala *et al*., 2019). These consequences and dangers prove that existing treatments are not effective enough to treat psoriasis, so this study was conducted to find safer alternative therapies for patient with psoriasis. *Physalis angulata L.,* known in Indonesia as "*ciplukan*," contains the active substance physalin, a derivative of steroids, flavonoids, and alkaloids (Nurfiana & Sari, 2018). The content of physalin B and F has been shown to have anti-inflammatory and anti-proliferation effects. The physalin mechanism inhibits lymphocyte proliferation, activation of macrophages, and proinflammatory cytokines through inhibition of the Nuclear Factor-kappaB (NF-kB) cascade (Zhang *et* al., 2012; Jacobo-Herrera *et al*., 2006).

MATERIALS AND METHODS

This research was a type of experimental research with randomized post-test only with control group design. The sample used was a female strain of BALB/c aged 2-3 months with a healthy weight of 20-25 grams. The mice was obtained from PHPM (Pengembangangan Hewan Percobaan Mandiri) in Yogyakarta. Thirty-five mice were randomized to be sampled in seven treatment groups. Group A only applied emollient in the form of Noroid cream®, up to 62.5 mg for seven days which had been shaved on the back, while Group B applied imiquimod cream 5% up to 62.5 mg (Aldara®; 3M Pharmaceuticals, UK) for seven days. Groups C to E received imiquimod cream 5% at a dose of up to 62.5 mg/kgBW for 14 days and *ciplukan* extract at 400, 800, and 1200 mg/kgBW from day 8 to day 14. Group F was the group that applied imiquimod cream 5% to 62.5 mg for 14 days and was given MTX (Ebewe tab 2.5 mg) at 1 mg/kgBW from day 8 to day 14 orally. Group G was the group that applied imiquimod cream 5% as much as

62.5 mg for 14 days and was given a combination of MTX 1 mg/kgBW and *ciplukan* extract 1200 mg/kgBW from day 8 to day 14 orally.

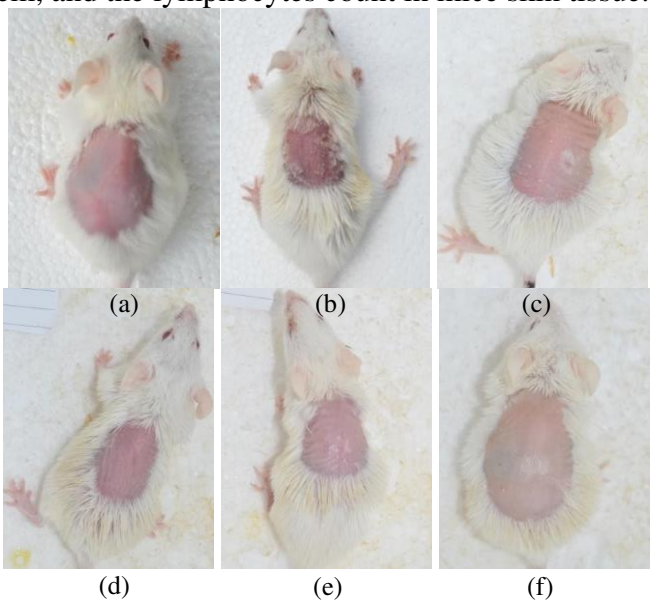
The manufacture of *ciplukan* extract using a multilevel maceration method with the first solvent was 96% ethanol, the second solvent was n-hexane, and the last solvent was ethyl acetate. The extract was made in the Pharmacology Laboratory and the Pharmaceutical Laboratory of UNSOED. The extraction results were also tested for liquid chromatography-mass spectrometry (LCMS-MS) test at the *Pusat Penelitian Kimia Lembaga Ilmu Pengetahuan Indonesia* (LIPI) Serpong, South Tangerang, Banten, and Thin Layer Chromatography Test at the UNSOED Pharmaceutical Laboratory.

Furthermore, sampling was conducted at the Pharmacology Laboratory of the Faculty of Medicine UNSOED. The skin tissue was cut on the subcutaneous back of the mice taken with a cross section. Mice skin samples that had been taken were made to block paraffin tissue with the coloring of Haematoxylin Eosin (HE) in the Anatomical Pathology Laboratory of the Faculty of Medicine, University of Sebelas Maret. The sample reading in the form of PASI score, Baker's score, and calculation of lymphocytes count of skin tissue in psoriasis mice model was conducted in the Laboratory of Pharmacology of the Faculty of Medicine UNSOED, assisted and guided by dr. Thianti Sylviningrum M.Pd.Ked, M.Sc, Sp.KK and Dr. dr. Dody Novrial, M.Si.Med, Sp.PA.

Univariate analysis was listed in the form of mean and standard deviation. Bivariate analysis used the Shapiro-Wilk test as a normality test and the Levene test as a homogeneity test. Furthermore, the Kruskal-Wallis non-parametric test was conducted for the PASI and Baker's score variables, while the lymphocyte count variable was analyzed using the One-Way ANOVA parametric test.

RESULTS AND DISCUSSIONS

The results of the study included PASI (Psoriasis Area Severity Index) score, Baker's scoring system, and the lymphocytes count in mice skin tissue.



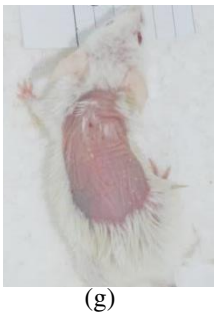


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Figure 1. Description of the PASI score of mice.
Experimental group; group A (a), group B (b), group C (c), group D (d), group E (e), group F (f), group G (g)

The PASI score was used to assess the severity of psoriasis disease while simultaneously evaluating the clinical picture of the course of psoriasis disease (Budiastuti, 2011). The PASI score index was modified for the mouse, namely erythema, squama/scales, and skin thickness (van der Fits *et al*., 2009). Kruskal-Wallis analysis found a value of p = 0.001 (p<0.05), which means there is a significant difference in at least two groups.

Table I. Results of the bivariate analysis of PASI skor scores

Groups	N	Mean ± Standard Deviation	*Shapiro Wilk*	*Levene's test*	*p value*
A	5	0,000 ± 0,000			
B	5	9,600 ± 2,302			
C	5	0,800 ± 0,447			
D	5	0,200 ± 0,447	<0,05	<0,05	<0,05
E	5	0,600 ± 0,548			
F	5	0,200 ± 0,447			
G	5	1,600 ± 1,140			

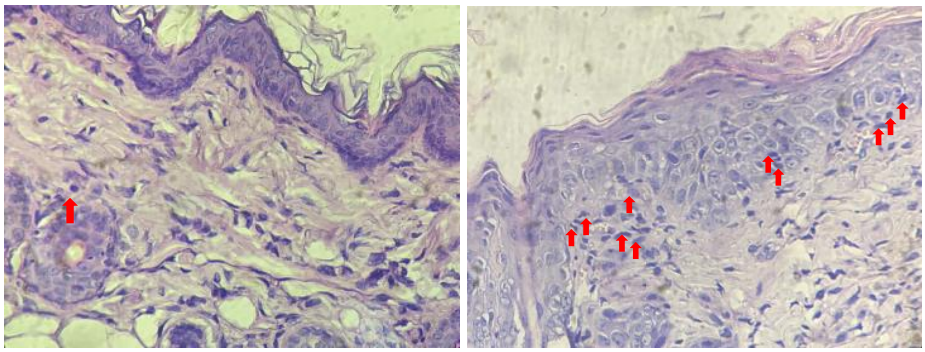
Table II. Results of the Baker's Scoring System bivariate analysis

Groups	N	Mean ± Standard Deviation	*Shapiro Wilk*	*Levene's test*	*p value*
A	5	0,000 ± 0,000			
B	5	5,000 ± 1,968			
C	5	2,800 ± 1,440			
D	5	2,600 ± 1,084	<0,05	>0,05	<0,05
E	5	1,300 ± 0,273			
F	5	1,800 ± 2,079			
G	5	3,100 ± 1,635			

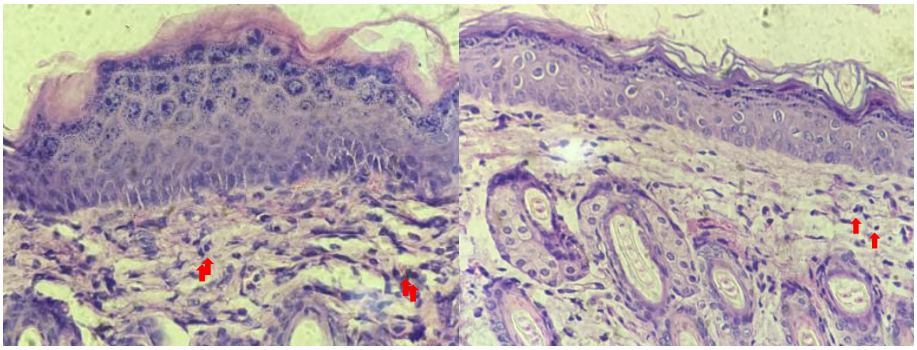
Baker's score was used to evaluate histopathological images of psoriasis with scales ranging from 0 to 10 (Baker *et al*., 1992). Kruskal-Wallis test results obtained a significance value of 0.001 (p<0.05). It shows that there is a significant difference in average Baker's score between groups.

Table III. Results of bivariate analysis of skin tissue lymphocyte count

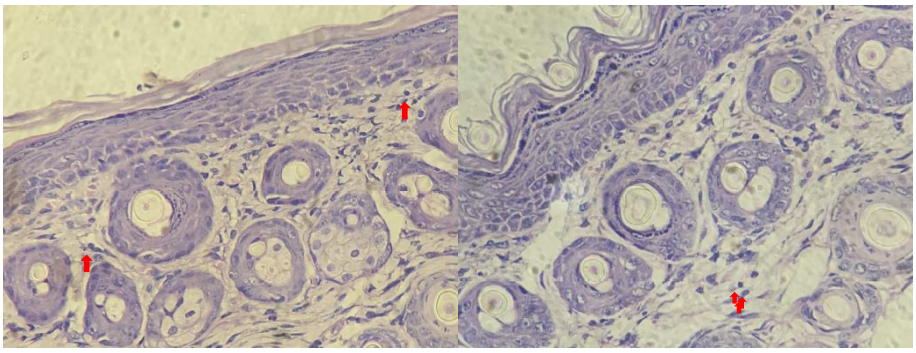
Groups	N	Mean ± Standard Deviation	*p value*	Description
A	5	0,240 ± 0,151	0,000	Terdapat perbedaan bermakna
B	5	0,958 ± 0,132		
C	5	0,526 ± 0,133		
D	5	0,326 ± 0,068		
E	5	0,437 ± 0,086		
F	5	0,335 ± 0,058		
G	5	0,720 ± 0,048		



(a) (b)



(c) (d)



(e) (f)

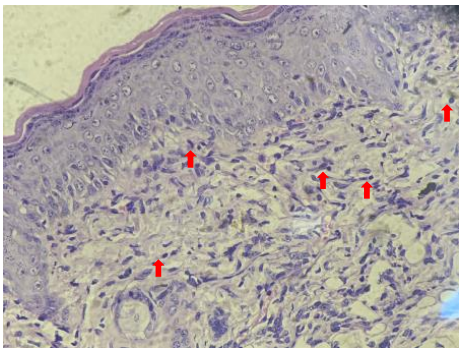


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Figure 2. Overview of histopathological preparations.
experimental group (lymphocytes are indicated by red arrows); group A (a), group B (b), group C (c), group D (d), group E (e), group F (f), group G (g)

One Way ANOVA test results on the lymphocytes count obtained a significance value of 0.000 ($p<0.05$). It suggests that there is a significant average difference in the lymphocytes count between groups. Group B, a sick control group with applied imiquimod for 14 days, showed a significant difference from the healthy group (Noroid cream®). It means that giving imiquimod (IMQ) could induce psoriasis features both clinically and histopathologically. The results are in line with Na Takuathung's study in 2018 which stated that giving IMQ for seven days can show significant improvements in PASI scores and mice histopathological images (acanthosis, epidermal hyperkeratosis, and inflammatory cell infiltration) (Na Takuathung *et al*., 2018).

The *ciplukan* extract group dose of 800 mg/kgBW and the MTX group of 1 mg/kgBW influenced the decrease in PASI score and the lymphocytes count comparable to group A. It means that *ciplukan* extract doses of 800 mg/kgBW had an effect comparable to the 1 mg/kgBW MTX drug on PASI scores and the lymphocytes count in skin tissue. The results support research conducted by Luliana in 2017, which stated that *ciplukan* herbal water extract has anti-inflammatory activity at a dose of 400 mg/kgBW, which also mentioned that the increased dose of *ciplukan* herbal water extract would increase anti-inflammatory effects (Luliana *et al*., 2017). These results are also in line with Zong's research in 2020, which stated that MTX can improve psoriasis lesions in the form of an erythema, thickening of the skin, and squama in male BALB/c mice when applied to imiquimod (IMQ) for seven days (Zong *et al*., 2020).

Active compounds include alkaloids, flavonoids, steroids/terpenoids, polyphenols, tannins, and saponins (Luliana *et al*., 2017). These active compounds will also affect the histopathological features of psoriasis. The content of physalin B is a secosteroid found in *ciplukan* extract that can inhibit the inflammatory response in macrophages by inhibiting NF-kB activation (Yang *et al*., 2018). The content of physalin F in *ciplukan* extract can inhibit the production of TNF-α, IL-6, IL-12, and NF-kB, which are key inflammatory factors in psoriasis (Ji *et al*., 2012; Jacobo-Herrera *et al*., 2006). It is contrary to the pathogenesis of psoriasis.

CONCLUSION

The present of the 800 mg/kgBW dose of ciplukan extract gives the most optimal effect in reducing PASI score and skin tissue lymphocytes count in psoriasis mice model that were not significantly different with the treatment control group, while 1200 mg/kgBW dose one gives the most optimal effect in reducing Baker's score that were significantly different with the treatment control group.

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