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The Effect of Aqueous Extract of Kalanchoe Folium on Methylprednisolone Pharmacokinetic Profile

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Abstract. Aqueous extract of *Kalanchoe pinnata* leaves had immunosupressant effect on lupus nephritis model. When it combined with methylprednisolone, there is a risk of interaction. In this study rats divided into two groups, a group that received methylprednisolone (MP) (0.72 mg/kgBW) and a group that received MP in combination with extract (0.36 mg/kg BW MP and 140 mg/kg BW extract). These treatment were given everyday for 4 weeks. Methylprednisolone concentration in rats serum was measured using HPLC with extraction method according to Lawson method (1985). The column used was Inertsil C-18 using mobile phase KH2PO4 : metanol (15:85) buffer, flow rate 0.6 mL/minutes, UV detector (λ = 230 nm) and pressure 1319 psi. The result showed that there was an interaction occurred. The combination of MP and aqueous extract of *K. pinnata* leaves showed interaction which causing methylprednisolone level comparable with methylprednisolone level in MP group. Mean of decreasing MP level in serum was 0.285 ppm. It means that aqueous extract of Kalanchoe Folium could elevate methylprednisolone concentration in plasma. Adjustment of the dose must be considered in this combination.

Keywords: *combination; interaction; K. pinnata; methylprednisolone; pharmacokinetic profile*

1 INTRODUCTION

Systemic Lupus Erythematosus (SLE) patients need a long term therapy with high risk[1,2]. Drug development for this condition is in progress. Some of them are cell-based therapy, monoclonal antibody and natural product medicine [3,2]. On the previous study, we had a result that aqueous extract of *K. pinnata* leaves could reduce glomerulonephritis severity in Balb/c mice treated by 2,6,10,14 tetramethylpentadecane (TMPD). Bergmann *et al* (1994) had studied an immunosuppressant activity of aqueous extract of *K. pinnata* leaves [4]. On previous study, this immunosuppressant activity is effective on lupus-like disease model treated by 2,6,10,14 tetramethylpentadecane (Pristane®). The active compounds reduce the severity of glomerulonephritis in mice model.

Compounds observed in *K. pinnata* were very complex[5]. One major compound that presented immunosuppressant activity on allergy model was quercetin 3-O-α-L-arabinopyranosil (1→ 2)- α-L rhamnopyranoside. The other compounds were quercetin 3-O-α-L-rhamnopyranoside, kaempferol 3-O-α-L-arabinopyranosil (1→ 2)- α-L rhamnopyranosida, and 4',5-dihydroxy-3',8-dimetoksiflavon 7-O-β-D-glukopiranosida[6,7]. The complexity of organic compounds in herbal medicine leads some interaction if it is given as a combination with conventional drug in therapy[8]. It might be considered in lupus therapy because the long-term maintenance therapy needs a high dose of corticosteroid (i.e. methylprednisolone) [1].

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2 Materials and Methods

2.1 Materials

The leaves of *Kalanchoe pinnata* (Lmk) Pers. were collected from Manoko, Bandung. The identification was confirmed at the Herbarium Bandungense, Bandung Institute of Technology,KH_2PO_4, methanol p.a ordered from chemical warehouse of School of Pharmacy, Bandung Institute of Technology, Male Wistar rats aged 3 months old ordered from Unit of Experimental Animal in Department of Pharmacology and Clinical Pharmacy Bandung Institute of Technology. Rats were housed under specific conditions. Rats were fed with standard diet and water ad-libitum

2.2 Methods

2.2.1 Extraction

The fresh leaves (3 kg) mixed with water, blended then the filtrates separated from the residue. This step was followed by filtration and lyophilisation. Then the dried powder was obtained. The aqueous extract was dissolved in a given volume of distilled water, giving rise to the stock solution (1g/mL) for subsequent use[4].

2.2.2 Measuring the Methylprednisolone Pharmacokinetic Profile

The animal model was three months old male Wistar rats (*Rattus norvegicus*). These rats divided into two groups that consist of 6 rats, a group that received methylprednisolone (MP) (0.72 mg/kgBW) and a group received MP in combination with extract (0.36 mg/kg BW MP and 140 mg/kg BW extract). These treatment were given everyday for 4 weeks. On the 29th day, the serum of all groups was taken at 1,3,5 and 24 hours after the last dose administered orally. Methylprednisolone concentration in rats serum was measured using High Performance Liquid Chromatography (HPLC) with extraction method according to Lawson method (1985)[9]. The column used was Inertsil C-18 using mobile phase KH2PO4 : metanol (15:85) buffer, flow rate 0.6 mL/minutes, UV detector (λ = 230 nm) and pressure 1319 psi.

3 Result and Discussion

K. pinnata fresh leaves were selected then the extraction method used is as the previous study[10]. The yield of dried extract was 2,92% w/w. Twelve rats were divided into two groups. The first group as the control group receive methylprednisolone 0.72 mg/kgBW. The second group receive a combined dose of methylprednisolone 0.36 mg/kg BW MP and 140 mg/kg BW extract. These treatment lasting for 4 weeks. This period based on the previous study. Proteinuria and glomerulonephritis severity were reduced by a three-weeks treatment of aqueous extract of *K. pinnata* leaves in a lupus model. The immunosuppressant active compound in *K. pinnata* that proven as anti inflammation and anti allergy was flavonoid, with a main flavonoid is quercetin 3-*O*-α-L-arabinopyranosil (1 → 2) α-L-rhamnopyranoside[11,12].

Before measuring pharmacokinetic profile, there is a need to calibrate the instrument. Instrument used in this research is High Performance Liquid Chromatography (HPLC). The calibration curves measured was the concentration of methylprednisolone in methanol and the concentration of methylprednisolone in rat plasma. The data presented in Table 1 and Figure 1.

Table 1 Calibration data of methylprednisolone in methanol

Concentration (ppm)	AUC	c kal
0.5	5491	-0.4642
1	19106	1.3352
5	46604	4.9694
15	127429	15.6513

35	276637	35.3709
50	388997	50.2205

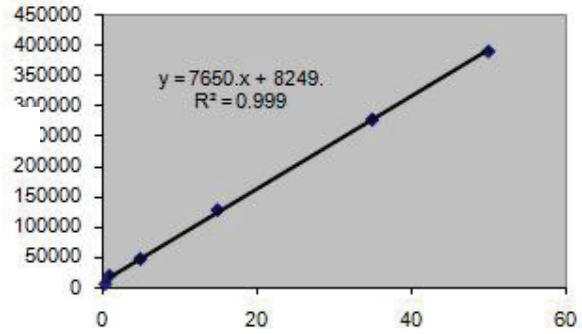

Figure 1 Calibration curve of methylprednisolone in methanol

This data is linear with R value 0,999. The calibration continued to the concentration in rat plasma. Table 2 and Figure 2 showed the data.

Table 2 Calibration data of methylprednisolone in rat plasma

Concentration (ppm)	AUC	c kal
0.5	174	0.9419
1	1346	1.0876
5	25922	4.1426
10	64897	8.9876
25	199459	25.7184

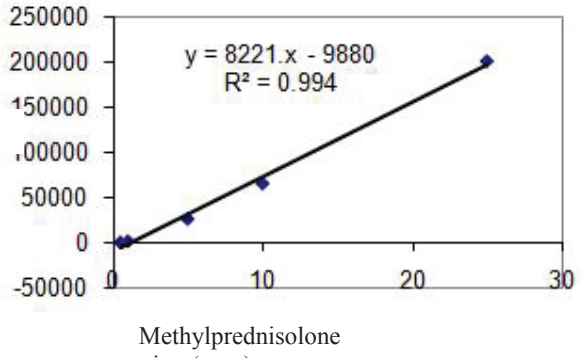

Figure 2 Calibration curve of methylprednisolone in rat plasma

The curve is linear with R value is 0.994 so it is used as standard for further measurements.

Pharmacokinetic profiles curve in both groups were similar. But we must remember that the treatment dose of methylprednisolone that was not the same. The combination group only received a half of another group. We can conclude the higher concentration in combination group. The highest concentration is in interval of 1 hour after treatment. The elevated concentration was not statistically significant ($p > 0,05$) in this experiment. In both group, methylprednisolone concentration decreased on interval 3 hour and 5 hour, then elevated on interval 24 hour. The data showed in table 3 and 4.

Table 3 The data of methylprednisolone concentration in group 1 (methylprednisolone only)

The interval of sampling (hours)	Mean of Cp methylprednisolone (ppm)
1	4.2057 ± 1.08
3	2.1167 ± 1.27
5	0.6628 ± 0.24
24	1.5498 ± 0.56

Table 4 The data of methylprednisolone concentration in group 2 (methylprednisolone in combination)

The interval of sampling (hours)	Mean of Cp methylprednisolone (ppm)
1	5.6057 ± 2.49
3	1.8861 ± 0.72
5	0.5392 ± 0.33
24	1.6457 ± 0.81

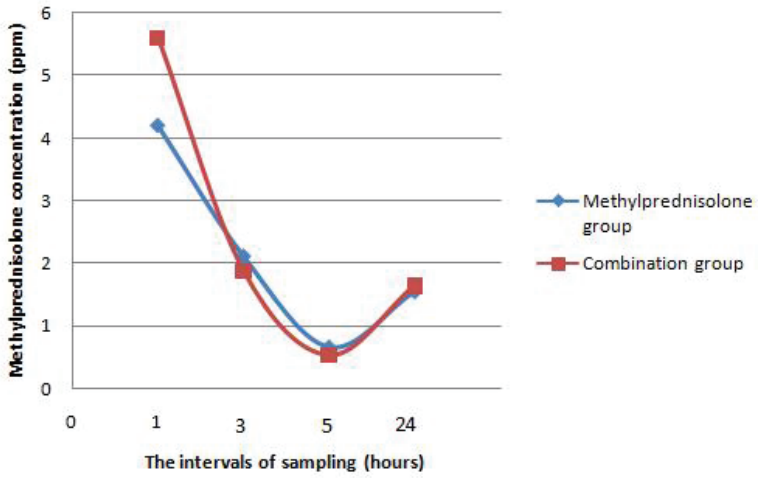

Figure 3 Pharmacokinetic profile of methylprednisolone in rat serum

Methylprednisolone dose used in combination treatment is a half of a group that received methylprednisolone only. In figure 3, the pharmacokinetic interaction occurred leads the elevated level of methylprednisolone in serum. This result might be occur because of metabolism process in the liver by cytochrome P450 enzyme. This enzyme has a limited capacity to metabolize drugs[13]. Based on the initial treatment of combination group, it is predicted that CYP450 metabolize compound in aqueous extract of *K. pinnata* leaves first. The impact is methylprednisolone can not bound to this enzyme. Methylprednisolone in unmetabolized form soluble in plasma then increase methylprednisolone level in blood.

The second hypothesis is the *K pinnata* organic compounds. Some of them might have a steroid structure. Beside flavonoid, there were compounds with contain steroid structure[5]. Flavonoids in *K. pinnata* produced a reduction of edema formation and its effectiveness was obtained using lower doses when compared to KPFE and was active from 0.3mg/kg[12]. These

findings are very promising considering that, recently, the anti-inflammatory activity of the leaf extract from *K. pinnata* was attributed to a novel steroid derivative, when Afzal et al.[14], using the model of carrageenan induced rat paw edema and oral administration, showed that the extract (400mg/kg) and the steroid (300mg/kg) reduced the inflammation by 87% and 84%, respectively. These authors[14] needed to administer a high dose of the steroid compound from the leaf extract to obtain approximately the same effect as our results using the flavonoid of *K. pinnata*. Furthermore, flavonoid of *K. pinnata* produce antiedematogenic effect at lower doses than those required to produce antinociceptive effects, suggesting that they are more effective to produce the antiedematogenic and anti-inflammatory effects than the antinociceptive effect, as observed to other extracts or isolated compounds[15-17]. These results support the hypothesis that the methylprednisolone pharmacokinetic profile changed in the presence of other steroid compounds in aqueous extract of *K. pinnata*. The further research is a need to ensure this result.

4 Conclusion

Aqueous extract of Kalanchoe Folium could increase methylprednisolone concentration in plasma. However, adjustment of the dose must be considered in this combination.

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Nomenclature

AUC	: Area Under the Curve
BW	: Body Weight
Cp	: concentration in plasma
k cal	: concentration in calibration
MP	: methylprednisolone
ppm	: part per million

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