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The effect of *Euphorbia milii* tea and its combination with *Propolis* on number of glomeruli in *M.tb*-infected mice: a histopathology study

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ABSTRACT

Background: The role of both *Euphorbia milii* and *Propolis* is to increase the effectiveness in pathological condition of the body part damaged, include renal function in maintaining the homeostasis. That combination into mice which are infected by *Mycobacterium tuberculosis* will change the total number of glomerulus in the kidney.

Aim: The study aims to determine the effect of combination tea from *Euphorbia milii* and *Propolis* on the number of the glomerulus in mice infected by *M. tb*.

Method: This study confirmed randomized post-test only control group design consisting of 24 mice divided into six groups (G1-G6). *E. milii* flowers are obtained from flower plantations, in Ketewel, Gianyar,

Bali Province took in fresh and dark red sampling. *M. tuberculosis* used H37Rv strain (ATCC27294) grown on Middlebrook 7H9 liquid medium for two weeks.

Result: It was found that Group 3 and Group 5 from the treatment group has the most significant average number of glomerulus which was 13, followed by Group 2 and Group 6 from the control group has the largest average number of glomerulus which was 12. The differences were not significant between groups with $P = 0.463$ ($P < 0.05$).

Conclusion: The *Euphorbia milii* tea and its combination with *Propolis* does not affect histological change on the total number of glomerulus in mice infected with *M. tb*.

Keywords: *Euphorbia milii*, *Propolis*, renal tuberculosis, glomerulus filtration rate

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INTRODUCTION

The *Euphorbia milii* which is also known as the Christ thorn is a species of flowering plants. It is originated from Madagascar. The parts of the *Euphorbia milii* that grows above the ground are used for medication. These are efficient for curing breathing disorders. Treatment for the mucus in the nose and throat. The latex component in the plant is moderately poisonous.¹ *Propolis* is a red or brown resinous substance which is made by mixing saliva and beeswax gathered from the tree buds, sap flows, or other botanical sources. *Propolis* is mainly used in cosmetic industries because of its high content of antioxidants, antibacterial, antifungal, and anti-inflammatory.²

Tea derived from the *Euphorbia milii* plant and *Propolis* from the bee. Both are combined and given to the mice. Later on, after a few months, the kidney of the mice is dissected and observed under the microscope to study the toxicity level due to the tea given. The role of both *Euphorbia milii* and *Propolis* is to increase the effectiveness in pathological condition of the body part damaged, associated with the influence of renal function as a vital organ in maintaining the body balance. Kidney function regulate body fluid balance by removing useless

or dangerous remnants of metabolism and keep substances that the body needs. This function is essential for the body to maintain homeostasis.³

The content contained in *Propolis* and *Euphorbia milii* also might get into the kidneys. Therefore the authors are interested in researching the effect of combination tea from *Euphorbia milii* and *Propolis* in mice infected with *M. tb* on kidney buds, number of glomeruli. Glomerulus is the parts going to be observed under the microscope in this research, because this part of kidney is the one that might get affected first by the substance used for this research, glomerulus works like a filter allowing only certain ingredients such a vitamin and mineral passed to the tubule, so this part of kidney is the front line for filtration system that might be affected first. Based on my opinion giving Combination tea from *Euphorbia milii* and *Propolis* to mice which are infected by *M.tb* will change the total number of glomerulus in the kidney.

METHODS

This study is experimental research with randomized post-test only control group design consisting of 24 mice divided into six groups (G1-G6). In G1, G3 and G5 were given an EMP

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dose of 15 mg / 20 grBW for 4 weeks while G2, G4 and G6 were given sterilized water. On the 28th day, *M. tb* H37Rv 60 µl infection (10^5 bacteria per ml) occurred in all groups. On days 29, 30 and 35 post-infection were terminated respectively on G1, G2 then G3, G4 last G5, G6. The taken kidney will be observed under the microscope to calculate the total number of glomerulus in the kidney.

Isolation and preparation of *M.tb* antigens were performed by default in the microbiology laboratory Sanglah Hospital. The preparation of *E. milii* and *Propolis* tea was performed at Faculty of Farming Technology, Udayana University. The phytochemical test of EMP tea was conducted at the Faculty of Mathematics and Scientific Science, Udayana University. Maintenance and treatment of mice and the histopathology study of the mice kidney were performed at Histology Laboratory, Medical Faculty, Udayana University.

Mycobacterium tuberculosis used H37Rv strain (ATCC27294) grown on Middlebrook 7H9 liquid medium for two weeks then stored at -70 °C ready for research. Before the bacterial suspension is performed thawing at 37°C and disonification for 10 seconds to dissolve the existing bacterial clumps so homogeneous and one cell separated from each other. Germs with a concentration of 105 per ml bacteria in 60 µl were inoculated intranasally using a micropipette.⁴

E. milii flowers are obtained from flower plantations, in Ketewel, Gianyar, Bali Province. It was took in a fresh and dark red condition. A total of 100 grams of *E. milii* flowers steamed at 100°C for 90 seconds. Then cooled for 5 minutes, dried in an oven at 95°C for 30 minutes, then ground flowers to powder. The powdered tea is sieved with a 120 mesh sieve. The honeycomb nest is obtained from Planta Badung honeybee plant, Bali. There were as many as 100 grams of honeybee that have been empty (tala) boiled with a temperature of 60 - 70°C until tala into a black liquid, and then on the surface will float the more whitish. This part of the cover is taken by absorbing it using filter paper and squeezed it back to obtain *Propolis*.

Tea combination of flower *E. milii* and *Propolis* prepared by brewing 100 gr tea powder *E. milii* flower in 200 ml of water after 5 minutes and then filtered. Then 100 gr of *Propolis* was stirred and ready to be given to the intervention group. Examination of kidney especially at glomerulus and part of the specimen that has been stained by HE examined under the microscope with 400 times magnification by using Optilab camera. Changes that will be observed in the experiment histopathological is the total number of the glomerulus in all groups in both control and treatment group.

The form of data obtained from histological changes in total number of the glomerulus in the kidney of mice infected with *M. Tb* will be arranged in table for later statistically analyzed using the *Kruskal-Wallis test*. The whole process of analysis done with SPSS 21 for Windows. The quantity data of glomerulus of the kidney histopathology in tabulation, tested normality and homogeneity of data if homogeneous and normal distributed data were continued with *Kruskal-Wallis* parametric test to check $p < 0.05$ or not.

RESULTS

It was found, that Group 3 and Group 5 from the treatment Group has the most significant average number of glomerulus which was 13, followed by Group 2 and Group 6 from the control group has the largest average number of glomerulus which was 12. The complete result for the data of total number of glomerulus can be shown in [table 1](#). The observation of the infected mice kidney was observed under a microscope with 400x magnification by using optilab camera.

In the G2-G6 group, data were normally distributed ($P > 0.05$), but in the G1 group, the data did not normally distribute $p = 0.06$ ($P < 0.05$) which can be used for the upcoming parametric test. The homogeneous data of the total number of glomerular is $p = 0.225$ ($P > 0.05$) so that it could proceed to the next parametric. Data is not normally distributed but is homogeneous, so comparison

Table 1 Characteristic of total number glomerulus for each sample

Group	T/C	Duration of termination after MTb Infection	Total Number of Glomerulus					Mean
			Sample 1	Sample 1	Sampl e 3	Sample 4	Sample S	
G1	Treatment	24 hours	9	0	0	0	9	0.4
G2	Control	24 hours	0	6	6	9	12	0.2
G3	Treatment	48 hours	13	11	8	0	9	9.8
G4	Control	48 hours	9	0	0	7	7	7.0
G5	Treatment	7 days	10	9	9	13	6	9.4
G6	Control	7 days	9	12	0	1	9	9

Table 2 Total number of glomerulus infected by *M. tb*.

Group	N	Mean	Std. Deviation	Std. Error	95% CI		Minimum	Maximum
					Lower Bound	Upper Bound		
G1	5	8.4	0.547	0.244	7.719	9.080	8	9
G2	5	8.2	2.489	1.113	5.108	11.291	6	12
G3	5	9.8	2.167	0.969	7.108	12.491	8	13
G4	5	7.8	0.836	0.374	6.761	8.838	7	9
G5	5	9.4	2.509	1.122	6.283	12.516	6	13
G6	5	9	1.870	0.836	6.677	11.322	7	12
Total	30	8.77	1.869	0.341	8.068	9.464	6	13

N, total number of samples; CI, Confidence Interval

Figure 1 total number of Glomerulus in Group 1 (Left), and in Group 2 (Right), are 8, respectively

Figure 2 total number of Glomerulus in Group 3 (Left) is 11, and in Group 4 (Right) is 8

Figure 3 total number of Glomerulus in Group 5 (Left) is 9, and in Group 6 (Right) is 8

not significant between groups with $P = 0.463$ ($P < 0.05$).

DISCUSSION

Microscopic observation of the histological changes at mice kidney was used to determine the effect of combination tea from *Euphorbia milii* and *Propolis* on kidney histology: number of the glomerulus in mice infected by *M. tb*. The main target of observations is total number of the glomerulus.

The result based on statistical analysis shows that the administration of combination tea from *Euphorbia milii* and *Propolis* does not affect the total number of glomerulus in mice kidney histopathology when compared both treatment and control group, the results show that glomerulus is normal. Apart from that, both treatment group that were given combination tea from *Euphorbia milii* and *Propolis* and control group that was given sterilized water does not show much significant mean difference. The result shows there no much effect on the total number of glomerulus, and the total number of glomerulus indicates that whether there is any degeneration of glomerular occurred or not. While we might find a bit of necrosis in tubules that mainly pycnotic, both of that result might have affected by the compound that *Euphorbia milii* and *Propolis* contain in this chase all the vitamin and mineral to help maintain the organ health but also stated that there some mineral that is considered as a toxic, *Propolis* contains resins such as flavonoids and other substances that include vitamins such as vitamin A, B1, B2, B6, C, D, E and trace minerals such as calcium, magnesium, iron, copper, zinc.⁵

The author did this research to know the safety of *Euphorbia milii* and *Propolis* for kidney and what other benefits from them in the kidney, from the result that shows combination tea from *Euphorbia milii* and *Propolis* does not change the total number of glomerulus in kidney histopathology, and a little bit of pycnotic visible in the histological profile,⁶

tests between groups use the Kruskal Wallis Test. In the Kruskal-Wallis test, the differences were

and explain that pycnotic seen at sites where there is evidence of inflammation generally indicates accidental cell death caused by some extrinsic hazard, such cell death termed necrosis.⁷ Cells can also perish under physiologically normal condition, for example, when they become senescent, so in this chase pycnotic that visible might not because the effect of *Euphorbia* and *Propolis* itself, instead its naturally occurred, the author make the conclusion that the combination tea from *Euphorbia milii* and *Propolis* is safe for the kidney and even can be beneficial supplement as the study says that the renal oxidative stress caused by doxorubicin can be prevented by the effect of flavonoid content in *Euphorbia* and *Propolis*, and as we all know *Euphorbia milii* and *Propolis* have a lot of benefits to our body and can be used for supplement.⁸ Supplementation is essential for the treatment of specific health problems but there is little evidence of benefit when used by those who are otherwise healthy.⁹ Therefore, treatment of combination tea from *Euphorbia milii* and *Propolis* to healthy mice does not affect the total number of kidney.^{10,11}

CONCLUSION

The *Euphorbia milii* tea and its combination with *Propolis* does not affect histological change on the total number of glomerulus in mice infected with *M. tb*. That combination of tea has a lot of benefits to our body and can be used for supplement to strengthen immune system and does not affect the total number of glomerulus in kidney histopathology.

CONFLICT OF INTEREST

The authors stated that there was no conflict of interest with regard this research.

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