



## THE EFFECT OF SNAIL MUCUS (*ACHATINA FULICA*) ON THE DEGREE OF KIDNEY INTERSTITIAL FIBROSIS IN LUPUS NEPHRITIS MICE MODEL

Arief Nurudhin<sup>1</sup>, Zainal Arifin Adnan<sup>1</sup>, Yulyani Werdiningsih<sup>1</sup>, Nurhasan Agung Prabowo<sup>1</sup>, Fathina Nisa Rabbani<sup>2\*</sup>

### Affiliation:

1. Rheumatology Division  
Department of Internal  
Medicine, Faculty of Medicine,  
Sebelas Maret University,  
Ketingan, Jebres, Surakarta,  
57126
2. Students of Internal Medicine  
Specialist Education Program,  
Faculty of Medicine, Sebelas  
Maret University, Ketingan,  
Jebres, Surakarta, 57126

Correspondence: Fathina Nisa  
Rabbani, fathinanisa@gmail.com,  
Student of Internal Medicine  
Specialist Education Program,  
Faculty of Medicine, Sebelas Maret  
University, Ketingan, Jebres,  
Surakarta, 57126

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### ABSTRACT

**Introduction:** cytotoxic drugs dominate SLE therapy, so new therapies with minimum side effects are needed. Snail (*Achatina Fulica*) mucus contains two types of protein; heparan and acharan sulfate. The snail mucus has significant potential benefits, but its use in lupus nephritis has never been examined.

**Methods:** This research used an experimental model. It used mice for the experiment. The mice were divided into five groups: control, untreated lupus, standard therapy (methylprednisolone), snail mucus therapy, and a combination of standard therapy and snail mucus. Renal histology was examined at the end of the fifth month. Data were analyzed using the Shapiro-Wilk, ANOVA, and Tukey Post Hoc tests.

**Results:** The level of interstitial fibrosis between the standard therapy group and the snail mucus group showed an MD value of -0.13 with a p-value of 1.00, meaning there is no significant difference between the level of interstitial fibrosis in the two groups. The comparison between the standard therapy group and the combination of the standard therapy group showed an MD value of 0.64 with a p-value of 0.992, which means that the combination of standard therapy tends to decrease the level of interstitial fibrosis.

**Conclusion:** The administration of snail mucus in combination with standard therapy affects the decrease in the level of interstitial fibrosis in mice model lupus.

**Keywords:** *Achatina fulica*; lupus nephritis; renal interstitial fibrosis; snail mucus.

## INTRODUCTION

Lupus nephritis (LN) is the most common cause of kidney damage, occurring in 40-60% of people with systemic lupus erythematosus (SLE). Kidney involvement is indicated by the presence of hematuria, proteinuria or decreased kidney function and requires renal biopsy. LN is a significant risk factor for overall mortality and morbidity in SLE [1,2].

The SLE is characterized by various autoantibodies that can form immune complexes that settle in the kidneys, contributing significantly to the pathogenesis of LN. The occurrence of LN is initiated by the role of the complement cascade, autoantibodies, intolerance, *cross-talk* adaptive and innate immune systems, recruitment of inflammatory cells, and finally, fibrosis which promotes kidney damage. Kidney inflammation is one of the most severe manifestations of SLE. It is characterized by autoantibody and complement deposition, cytokine production, activation and recruitment of inflammatory cells, and damage to microvascular and parenchyma in the kidneys. A study proved that interstitial fibrosis, renal vascular SLEions and glomerular crescent in LN patients are reliable predictors of renal prognosis [3,4].

Snails (*Achatina fulica*) are animals with a large abundance of species in Indonesia that have many benefits, including snail mucus. Snail mucus functions as an antibacterial and anti-inflammatory, which can accelerate the inflammatory phase so that the proliferative phase will be faster in wound healing. *Achatina fulica* mucus contains two types of proteins that have an essential role in the body's metabolism. Heparan sulfate given from the outside is also thought to replace glomerular membrane filtration damaged by immune complexes in lupus nephritis [5-7].

The primary therapy in SLE disease is still dominated by cytotoxic drugs, so more effective therapy is needed with milder side effects in treating lupus nephritis. Research continues to search for new therapeutic strategies with higher effectiveness and lower comorbidities. This study aims to find alternatives to existing drugs to prevent progressivity and improve the quality of life of people with lupus nephritis with minimal side effects and low prices. The use of snail mucus (*Achatina fulica*) in the SLE and lupus nephritis has never been done, which has the advantage of a low price and excellent benefits in overcoming inflammation.

## METHOD

### Type and Design of Research

This study is an experimental research with mice as experimental animals. The place of maintenance, induction of animals, and the manufacture of histological preparations is carried out at the Histological and Biomedical Laboratory of the Faculty of Medicine, Sebelas Maret University, Surakarta.

### Research Subjects

The subjects were male mice of *Mus musculus* subspecies strain Balb / C aged 3-4 months, weighing 20-30 grams provided by the Faculty of Veterinary Medicine, Universitas Gadjah Mada. Mouse foodstuffs are used as standard mouse feed BR I.

The selection of mice is based on the consideration that *Mus musculus* mice are most often used in biomedical research because they are genetically similar to humans and can adapt to a laboratory environment.

### Sampling Technique

Balb / c male mice aged 3-4 months with a body weight of 20-40 grams were taken as many as 35 heads randomly by simple random sampling method and then divided into five groups, namely:

1. Control group: NaCl 0.9% 0.5 ml intraperitoneally administered on the first day of treatment.
2. Group lupus nephritis: Pristan 0.5 ml intraperitoneally administered on the first day of treatment.
3. Group lupus nephritis with standard therapy: Pristan 0.5 ml intraperitoneally administered on the first day of treatment and methylprednisolone (MP) dose 5 mg per kg orally per day at months 4 and 5.

4. Group lupus nephritis with snail mucus therapy: Pristan 0.5 ml intraperitoneally administered on the first day of treatment, 0.5 ml oral snail mucus at months 4 and 5.
5. Group lupus nephritis with standard therapy and snail mucus: Pristan 0.5 ml intraperitoneally administered on the first day of treatment, MP dose 5 mg per kg oral per day and 0.5 ml oral snail mucus at months 4 and 5.

At the end of the experiment, mice were sacrificed after treatment to take kidney tissue samples. The preparation is observed histologically, and the degree of renal tissue with interstitial fibrosis is calculated. Calculation of intercolonial fibrosis as a per cent of the total tissue occupied by fibrous tissue after the trichrome painting was previously done. Observation of renal histopathological preparations using a light microscope in the field with weak magnification (100x) and strong magnification (400x).

#### Data Analysis Methods

Steps analyzed the data obtained: descriptive analysis, normality analysis with the Shapiro-Wilk test with average distribution results, and then the F Anova test was used at the level of meaning  $\alpha = 0.05$  continued by looking for differences of 2 means between sample groups for each variable using a follow-up test, namely the Tukey Post Hoc Test.

#### Ethical Clearance

Treatment of experimental animals is carried out following ethical clearance. The procedure for causing lupus in mice is done by intraperitoneal injection of 0.5 ml pristan following several previous studies and journals.

This study was intended to determine the effect of *Achatina fulica* mucus therapy on the degree of interstitial fibrosis in the kidneys of mice with pristane-induced lupus nephritis. Before the hypothesis test was carried out, the description of the research variable was first explained, namely the degree of interstitial fibrosis in the control group, lupus nephritis, lupus nephritis with standard therapy, lupus nephritis with *Achatina fulica* mucus therapy, lupus nephritis with standard combination therapy of *Achatina fulica* mucus.

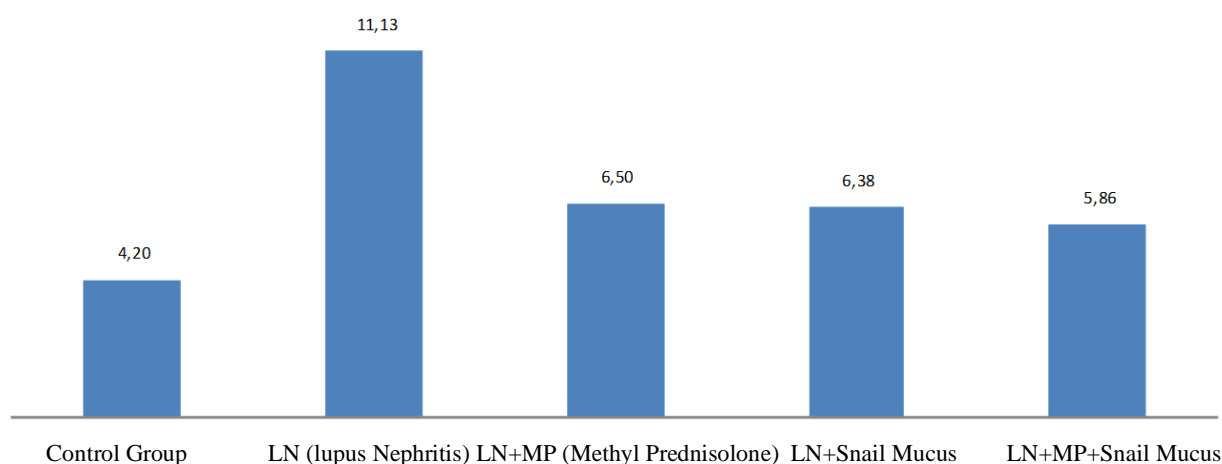
Table 1. Description and Variable Normality Test Degree of Renal Interstitial Fibrosis (%)

Group	Mean $\pm$ SD	Normality Test	
		Stat-SW	Itself
1. Control	4,20 $\pm$ 3,12	0,899	0,211
2. LN	11,13 $\pm$ 3,76	0,899	0,283
3. LN + MP	6,50 $\pm$ 1,93	0,892	0,244
4. LN+snail mucus <i>Achatina fulica</i>	6,38 $\pm$ 1,85	0,891	0,240
5. LN+ MP + snail mucus <i>Achatina fulica</i>	5,86 $\pm$ 2,73	0,936	0,602

Source: Primary Data 2021, processed.

Based on the variable description of the degree of renal interstitial fibrosis above, it can be seen that pristane-induced mice (mice model of lupus nephritis) have a higher average degree of renal interstitial fibrosis than in the control group. Giving MP in combination with *Achatina fulica* mucus can reduce the degree of renal interstitial fibrosis. The difference in the average degree of renal interstitial fibrosis between sample groups can be described as follows:

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Thus, the variable data distribution of the degree of renal interstitial fibrosis has been described briefly. Normality data testing has been carried out on these variables, and the results are that all research variables are normally distributed.

The first step tests the variation or difference in average k based on the sample group for the degree of renal interstitial fibrosis. The variable data distribution of the degree of renal interstitial fibrosis of all sample groups normally is distributed, then testing the variation or difference in the average of each group using ANOVA or test F. The results of ANOVA testing for the degree of renal interstitial fibrosis are as follows:

Table 2. ANOVA Mean Difference in Degree of Interstitial Fibrosis Based on Sample Group (%)

Group	Mean $\pm$ SD	ANOVA/ F Test	
		F Count	Itself
1. Control	4,20 $\pm$ 3,12		
2. LN	11,13 $\pm$ 3,76		
3. LN+MP	6,50 $\pm$ 1,93	7,210	0,001**
4. LN +Snail mucus	6,38 $\pm$ 1,85		
5. LN + MP + Snail mucus	5,86 $\pm$ 2,73		

Source: Primary Data 2021, processed.

Information:

\* Significant at 5 per cent Degree of Significance.

\*\* Significant at 1 per cent Degree of Significance.

The results of Anova or analysis of variations in the five means above show that the difference in 5 mean degrees of renal interstitial fibrosis produces a calculated F value of  $F = 7.210$  with a significance level of  $p = 0.001$  which means that the difference of 5 means is significant or convincing with a degree of significance of 5 per cent ( $p < 0.05$ ). This means that the mean variation in the degree of renal interstitial fibrosis in the control group, LN group, LN group with MP therapy (LN + MP), LN group with *Achatina fulica* mucus therapy (LN + *Achatina*

fulica mucus), and MP therapy combination group with *Achatina fulica* mucus (LN + MP + *Achatina fulica* mucus) was utterly different conclusively.

When compared with the average degree of renal interstitial fibrosis in the control group, the LN group has a tendency to have a higher average degree of interstitial fibrosis (increased) than the average degree of interstitial fibrosis in the LN + MP + *Achatina fulica* mucus group has the lowest average compared to other lupus nephritis groups or means that the degree of interstitial fibrosis can be suppressed, especially by administering MP combination therapy With mucus *Achatina fulica*.

The results of tracing the difference between two average degrees of interstitial fibrosis between sample groups can be explained by the following table:

Table 3. Tracing the Difference Between Two Variable Means of Interstitial Fibrosis between Sample Groups Due to Snail Mucus Administration

Search Difference 2 Mean Group	MD	Significance
Control – LN	-6,93	0,001**
LN – LN+MP	4,63	0,017*
LN – LN+ <i>achatina fulica</i>	4,75	0,014*
LN – LN+MP + <i>achatina fulica</i>	5,27	0,007**
LN+MP – LN+ <i>achatina fulica</i>	0,13	1,000
LN+MP – LN+MP+ <i>achatina fulica</i>	0,64	0,992
LN+ <i>achatina fulica</i> – LN+MP+ <i>achatina fulica</i>	0,52	0,996

Source: Primary Data 2021, processed.

Information:

\*) Significant at 5 per cent degree of significance.

\*\*\*) Significant at 1 per cent significance degree

The results of the analysis of the difference between two on average independent samples using *the Tukey Post Hoc Test* search above showed that the test on the degree of renal interstitial fibrosis between the LN + *Achatina fulica* mucus group and the LN + MP + *Achatina fulica* mucus group obtained a mean difference value of 0.52 with a probability of  $p = 0.996$ . This means that there was no significant difference between the mean degree of interstitial fibrosis in the LN+mucus group of *Achatina fulica* and the LN+MP+mucus group of *Achatina fulica* in the degree of significance of 5 per cent ( $p > 0.05$ ). The analysis results can explain that the combination therapy with MP and *Achatina fulica* mucus cannot improve conclusively the degree of renal interstitial fibrosis that has been given *Achatina fulica* mucus therapy alone.

Thus the research hypothesis stating that: "Snail mucus administration (*Achatina fulica*) decreased the degree of renal interstitial fibrosis in the mouse model of lupus nephritis" can be conclusively proven. The statistical analysis results can explain that with *Achatina fulica* mucus therapy, the degree of renal interstitial fibrosis can be conclusively reduced. Combination therapy of methylprednisolone with *Achatina fulica* mucus can also significantly reduce the degree of renal interstitial fibrosis in lupus nephritis mice.

## DISCUSSION

This study obtained a process of disease process stages, where intraperitoneal injection of pristane in BALB / c mice can develop a local inflammatory response and induce the production of autoantibodies that manifest clinically SLE. Pristan-injected mice exhibit clinical manifestations of SLE, including renal interstitial fibrosis. Renal interstitial fibrosis is a condition of excessive buildup of type-1 collagen in the renal interstitial, especially around fibroblasts and proximal tubules, a marker of decreased kidney function due to chronic inflammation and tissue injury. Renal fibrosis is a common feature of chronic inflammatory disorders in which the wound healing process persists and becomes redundant, with prolonged production of *transforming growth factor* (TGF) –  $\beta$ , fibrogenic cytokines, and proteolytic and inhibitory enzymes, leading to increased synthesis and decreased degradation of the extracellular matrix. The occurrence of damage to kidney tissue in this study can be detected at the known cellular level through histopathological examination [8,9].

Pristan i.p. injection will activate  $\text{NF}\kappa\beta$  and p38 MAPK so that there is an increase in TGF- $\beta$  production. TGF- $\beta$  will stimulate target cells, namely fibroblast cells, mesangial cells, podocytes, tubule cells and endothelial cells. Activation of these target cells will trigger the formation of ECM. Fibroblast cells will express type-I collagen and eventually cause kidney interstitial fibrosis. After intraperitoneal injection of pristan, caspase-1 production increases significantly, mainly produced by macrophages. It will also stimulate local  $\text{TNF}\alpha$ , causing the endothelium to express the e-selectin needed to bind to PMNs. The PMN will then express the MMP-9. MMP-9 further degrades collagen expressed by fibroblast cells. Under normal circumstances, according to the law of homeostasis, a balance of influence between TGF- $\beta$ 1 and MMP-9 occurs. TGF- $\beta$ 1 also inhibits MMP-9 expression expressed by PMN. In this study, pristane injection is expected to cause TGF- $\beta$ 1 to be more dominant than MMP-9, resulting in interstitial fibrosis. IL-1 $\beta$  will stimulate the endothelium to express ICAM. ICAM will bind monocytes. Then monocytes will enter the tissue and turn into macrophages. Increased macrophages will cause increased cytokine and caspase-1 expression processes resulting in increased fibrosis and kidney damage [10,11].

*Achatina fulica* contains two essential types of proteins, namely acharan sulfate and heparan sulfate. The administration of *Achatina fulica* mucus in this study aims to replace the filtration of glomerular sulfate membranes that immune complexes in lupus nephritis have damaged, reduce *fibroblast growth factor* (FGF) signals, and increase fibroblast proliferation. Also, it Enhances dendritic immature cell maturation, angiogenesis, and VEGF inhibition. So that by giving *Achatina fulica* mucus, it is expected that cell damage will be reduced, as a result of which stimulation of macrophages through TLR-4, TLR-7 and TLR-9 is also reduced. As a result, the expression of caspase-1 by macrophages is also reduced. This condition will play a role in correcting the imbalance state [9,12,7,13].

Necrotic cell death is characterized by swelling of the cytoplasm and organelles, followed by loss of integrity of the cell membrane and release of cell contents into the surrounding extracellular space, resulting in an inflammatory response of tissues. This will also induce the occurrence of  $\text{NF}\kappa\beta$  Activation, which will increase the production of growth factors, including TGF- $\beta$ 1. TGF- $\beta$ 1 will stimulate target cells, namely fibroblast cells, mesangial cells, podocytes, tubule cells and endothelial cells. Activation of these target cells will trigger the formation of ECM. Fibroblast cells will express type-I collagen and eventually cause kidney interstitial fibrosis [10,11].

Overall, this study showed a nephroprotective effect of *Achatina fulica* mucus where the nephroprotective effect was seen both cellularly (degree of interstitial fibrosis). Although there are currently no studies using *Achatina fulica* mucus in kidney disorders, the results of this study are reinforced by other studies that use components present in *Achatina fulica* mucus against other organ disorders, where Ferreras et al. administered Heparin Binding EGF-like growth factor (HB-EGF) and proved protective in the development of liver fibrosis. Another study in wild mice treated with the small molecule antagonist HS (bis-2-methyl-4-amino-quinolyl-6-carbamide, 1 mg/kg/day) for seven days after myocardial infarction provided improved heart function and survival [14,15].

Based on the principles of axiology, this study's overall benefit is that administering *Achatina fulica* mucus in mice with lupus nephritis can prevent/reduce the degree of fibrosis in the kidneys. *Achatina fulica* mucus is a compound that contains hyaluronic acid compounds, glycoprotein enzymes, proteoglycans and glycosaminoglycans. Glycosaminoglycans are a type of carbohydrate that plays an essential role in maintaining and maintaining connective tissue between cells. Heparan sulfate serves as a factor affecting cell division. In addition, this substance also serves as a protein attaching that serves as a signal for cell division stimulus for its receptors in the cell membrane. The addition of a concentration of heparin sulfate concentration absorbed by tissues will increase the proliferation of fibroblasts. Cell proliferation in injured tissue begins in the presence of FGF. Heparan sulfate also serves for angiogenesis, inhibition of *vascular endothelial growth factor* (VEGF) or decreased mitogen activity of FGF. Heparan sulfate, one of the proteoglycans, is a binder and *reservoir* for the *basic fibroblast* growth factor (bFGF) growth factor secreted into the Extra Cellular Muscular (ECM). ECM can release bFGF, which will stimulate inflammatory cell recruitment, fibroblast activation and the formation of new blood vessels with each injury, which in this study, the result is repair or inhibition of tissue damage or curtailing interstitial fibrosis in the kidneys of LN mice given *Achatina fulica* mucus [12,16,17].

Thus, administering *Achatina fulica* mucus in lupus nephritis can reduce damage to the kidneys, resulting in a decrease in the degree of renal interstitial fibrosis.

## CONCLUSION

*Achatina fulica* mucus (snails) has been shown to reduce the degree of interstitial fibrosis in mice with pristane-induced lupus nephritis.

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## CONFLICT OF INTEREST

The author declares there is no conflict of interest.

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