Original article

The Effect of Snail Mucus (Achatina Fulica) Toward The Activity and Chronicity Indices of Renal Histology in Pristane-Induced Lupus Nephritis Mice Model

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Abstract:

Background: Snail mucus (Achatina Fulica) has been known to contain heparan sulfate, and acharan sulfate, a glycosaminoglycan with anti-inflammatory properties and might repair the injured glomeruli. ***Aim:*** This study aims to find the effect of snail mucus on activity and chronicity indices of renal histology in a pristane-induced lupus nephritis mice model. ***Method:*** This was an experimental study with a post-test-only control group design using 42 mice strains Balb/C. Mice were randomized into six groups: control, lupus nephritis, methylprednisolone, snail mucus 0.5 ccs, snail mucus 0,5 cc and methylprednisolone, and snail mucus one cc and methylprednisolone group. ***Result:*** Snail mucus significantly affected the activity index (p = 0.001). The lowest activity index was shown by snail mucus one cc and methylprednisolone group. While the chronicity index was not significantly affected by the snail mucus with p = 0.195. Snail mucus decreases the activity index of renal histology in the pristane-induced lupus nephritis mice model but not the chronicity index.

Keywords: Achatina fulica; activity index; chronicity index; lupus nephritis; snail mucus.

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Introduction:

Systemic lupus erythematosus (SLE) is a common autoimmune rheumatic disease with a complex mechanism and pathogenesis [1-5]. SLE can affect the kidney, resulting in nephritis due to the presence of the immune complex in the glomerular basement membrane (GBM) [3,6,7]. Nephritis lupus is the most common and severe manifestation found in SLE, with the prevalence as high as 30-82% in Asia Pacific. Kidney involvement predicts morbidity and mortality in SLE [(3,8,9)]. The gold standard in diagnosing lupus nephritis is kidney biopsy. From the histological pattern, activity and chronicity indices can be measured to predict the prognosis and response to treatment [3,10].

Current SLE therapy uses immunosuppressive agents

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and steroids to inhibit the progressivity and severity of the disease. Those agents may increase the risk of morbidities, such as infection, osteoporosis, or cardiovascular and reproductive effects [11]. Thus, many studies were conducted to find therapy for SLE with minimal side effects.

Achatina fulica is a snail which quickly found in Indonesia. Its mucus contains heparan sulfate and acharan sulfate, which have been known to play an inflammatory response role[12]. Extrinsic heparan sulfate is also considered beneficial in replacing the damaged GBM due to the immune complex deposition in lupus nephritis[13].

A study using snail mucus (*Achatina fulica*) as an adjunctive treatment for lupus nephritis has never been conducted before. Snail mucus has excellent potential for use, considering its low cost and properties. Thus, this study aims to determine the effect of snail mucus on the activity and chronicity indices of renal histology in pristane-induced lupus (PIL) nephritis mice models.

Methods

The study was an experimental animal design using 42 male mice (*Mus musculus*) strain Balb/C, aged four months, weighed 30 grams. Mice were randomized into six categories. The pristane-induced lupus nephritis mice models were obtained intraperitoneally with 0.5 cc pristane.

The groups in this study were as follows: NC (negative control): saline 0.5 ccs intraperitoneally on before treatment, LN (positive control/lupus nephritis group): Pristane 0.5 ml on the first day of treatment, T1: Pristane 0.5 ml on the first day of therapy and methylprednisolone 5 mg/kg/day given orally for two weeks, T2: Pristane 0.5 cc on the first day of treatment, 0.5 cc/day of snail mucus given orally for two weeks, T3: Pristane 0.5 cc on the first day of therapy, methylprednisolone 5 mg/kg/day + 0.5 cc /day of snail mucus given orally for two weeks, and T4: Pristane 0.5 cc on the first day of treatment, methylprednisolone 5 mg/kg/day + 1 cc/day of snail mucus given orally for two weeks).

At the end of the study, a histopathological examination was conducted. Activity and chronicity indices were scored based on the total of each histological characteristic. Data were analyzed using the *Kruskal-Wallis* and *Mann-Whitney* tests, with Significance $p<0.05$.

Ethical clearance

This research was undertaken with the agreement of the Health Research Ethics Committee, Faculty of Medicine, Sebelas Maret University, under protocol number 059/UN27.06.6.1/KEPK/EC/2020.

Results:

From the histopathological examination, activity and chronicity indices can be scored. Activity index includes six characteristics: endocapillary hypercellularity, neutrophils/ karyorrhexis, hyaline deposits/wire loops, fibrinoid necrosis, cellular or fibro cellular crescents, and interstitial inflammation. At the same time, the chronicity index includes four characteristics: Glomerulosclerosis, crescents of fibrous tissue, tubular atrophy, and interstitial fibrosis on a global scale.

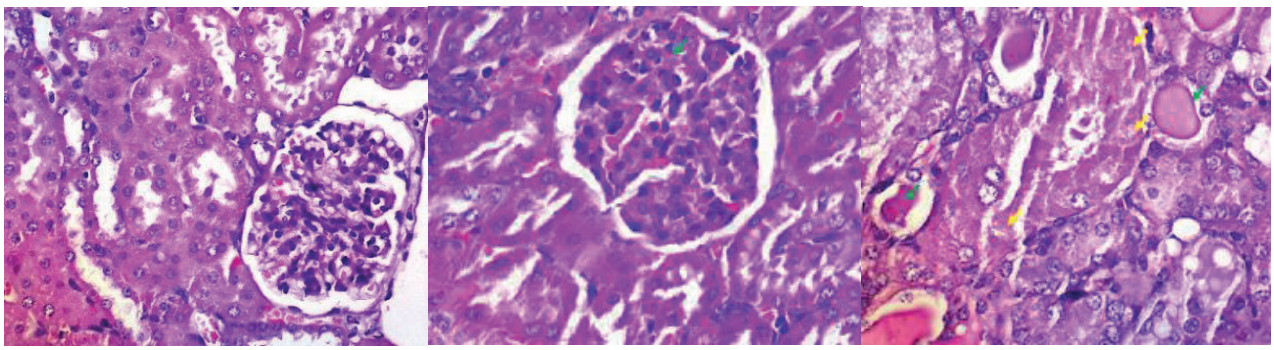

Figure 1. Histopathological Examination of Renal Histology. Hematoxylin-Eosin staining. 40x. 100x magnification. A: Healthy mice: Glomerular appearance is relatively normal; B: PIL mice: Infiltration of inflammatory cells and karyorrhexis (green arrow); C: PIL mice: Tubular atrophy (green arrows) and degenerative tubular epithelial (yellow arrows) are seen.

Based on the obtained scores, all the activity indices of treatment groups were lower than the KP (5.29±0.49). The lowest activity index between the treatment groups was shown by the P2 (3.86±0.69), followed by P4 (4.00±1.41), P3 (4.14±0.69), and P1 (4.71±0.95). From the *Kruskal Wallis* test, snail mucus was shown to significantly affect the activity index of renal histology with a p-value of 0.001. The data are summarized below.

Table 1. Descriptive and Comparative Analysis of Activity Index Variables

Group	Mean ± SD	Normality Test
KN	3.14 ± 0.38	<0.001
KP	5.29 ± 0.49	<0.001
P1	4.71 ± 0.95	0.183
P2	3.86 ± 0.69	0.099
P3	4.14 ± 0.69	0.099
P4	4.00 ± 1.41	0.006

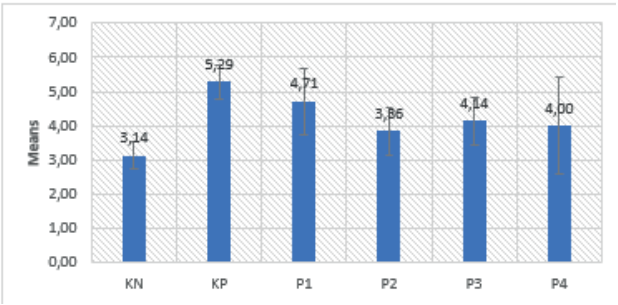

Figure 2. Bar Diagram of Activity Index Variables

The analysis continued with the *Mann-Whitney* test. From the result, the treatment groups that showed a significantly different score of activity index with KP were P2 (p = 0.003), P3 (p = 0.007), and P4 (p = 0.021), and all those three groups used snail mucus. The result also showed no significant difference between all the treatment groups (P1, P2, P3, and P4) with p > 0,05.

Table 2. *Mann Whitney* Test of Activity Index Variables

Group	p
CG vs. LN	0.001*
CG vs. T1	0.006*
CG vs. T2	0.035*
CG vs. T3	0.010*
CG vs. T4	0.096
CG vs. T1	0.203
CG vs. T2	0.003*
CG vs. T3	0.007*
CG vs. T4	0.021*
T1 vs T2	0.070
T1 vs T3	0.172
T1 vs T4	0.128
T2 vs T3	0.431
T2 vs T4	0.726
T3 vs T4	0.333

Notes: CG : control group; LN: Lupus nephritis group; T1: Methylprednisolone 5 mg/kg BW/day; T2: snail mucin 0.5 cc/day; T3: MP 5 mg/kg BW/day + snail mucin 0.5 cc/day; T4: MP 5 mg/kg BW/day + snail mucin 1 cc/day;

The data obtained from the lowest score of the chronicity index were as follows: KN, P1, P2, and P3 showed the same score (1.00±0.00), followed by P4 (1.14±0.38), and lastly, KP (1.29±0.49). From the *Kruskal Wallis* test, snail mucus was not shown to significantly affect the chronicity index of renal histology, with a p-value of 0.195. Thus, the *Mann-Whitney* test was not conducted. The data are summarized below.

Table 3. Descriptive and Comparative Analysis of Chronicity Index Variables

Group	Mean ± SD	Normality Test
CG	1.00 ± 0.00	<0.001
LN	1.29 ± 0.49	n/s
T1	1.00 ± 0.00	n/s
T2	1.00 ± 0.00	n/s
T3	1.00 ± 0.00	n/s
T4	1.14 ± 0.38	<0.001

Notes: CG: control group; LN: Lupus nephritis group; T1: Methylprednisolone 5 mg/kg BW/day; T2: snail mucin 0.5 cc/day; T3: Methylprednisolone 5 mg/kg BW/day + snail mucin 0.5 cc/day; T4: Methylprednisolone 5 mg/kg BW/day + snail mucin 1 cc/day; n/s: not specified (no data variance)

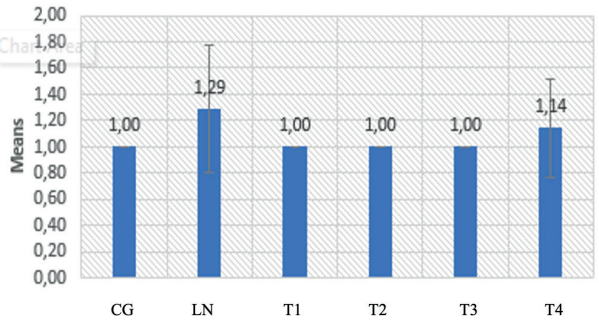

Figure 3. The bar Diagram of Chronicity Index Variables shows improvement of Chronicity Index Variables after being given snail mucin and methylprednisolone

From these results, it can be concluded that snail mucus (*Achatina fulica*) significantly reduced the activity index, but not with chronicity index, in pristane-induced lupus nephritis mice models.

Discussion:

Lupus nephritis is a glomerulonephritis that is commonly found in SLE. The pathophysiology of lupus nephritis is associated with the systemic and local/intrarenal immune response[14]. Lupus nephritis affects the glomeruli, tubules, and surrounding interstitial tissue. Glomeruli have been known to be responsible for the filtration process. GBM is mainly composed of heparan sulfate proteoglycans (HSPG) that contains heparan sulfate. The deposition of the immune complex might cause the loss of heparan sulfate, thus leading to impaired glomerular function[15].

A study by Harti et al., showed that snail mucus contains achatina isolate, heparan sulfate, and calcium, which is the most potent component to accelerate the wound healing process[16]. A pristane-induced lupus mice model is used to evaluate the potential of snail mucin as adjunctive therapy in lupus nephritis. This is due to a previous study that showed mice injected with pristane would develop lupus nephritis after 4-6 months[17].

Activity and chronicity indices are used to assess renal histology. The activity index represents the active lesions that might be responsive to immunosuppressive therapy. In contrast, the chronicity index refers to chronic kidney damage, which comprises refractory lesions after receiving aggressive therapy[18].

In this study, snail mucus *Achatina fulica* proved to have a significant effect on reducing the activity index with a p-value of 0.001. All treatment groups showed a lower activity index than the positive control group. P2, which only received snail mucin, led to the lowest activity index among the treatment groups. However, in general, all the treatment groups have similar efficacy.

In a previous study, the activity index had been shown to correlate positively with C3 complements[3,19]. The result follows the theory of complement system involvement in kidney tissue injury of lupus nephritis. Alternative pathway in complement activation causes the amplification loop that accelerates the cleavage of C3 into C3b, covalent binding onto cell surfaces, and release of anaphylatoxins C3a and C5a, also the formation of complement membrane attack complex. Complement activation is also responsible for tubular damage[20].

Snail mucin *Achatina fulica* contains heparin sulfate, which has been known to bind with various compliments. Zaferani et al., stated that heparan sulfate could bind complement, thus inactivating the complement cascade[21]. In the alternate route, heparan sulfate inhibits the complement cascade by binding factor H to its surface, hence speeding C3b inactivation, or it activates the cascade by binding stabilizing factor properdin to the apoptotic cell. Heparan sulfate is also essential for direct signaling via TLR-4, phagocytosis, and controlling the interaction between inflammatory mediators and their receptors. In angiogenesis, heparan sulfate will inhibit VEGF and decrease the mitogenic activity of FGF[22].

In lupus nephritis, where kidney tissue injury is found, extrinsic heparan sulfate supplementation might be beneficial to replace the filtration membrane on damaged GBM. This has been previously shown in a study in which snail mucin *Achatina fulica* successfully suppresses the antibody anti-dsDNA in pristane-induced lupus nephritis mice models[23].

Snail mucin *Achatina fulica* also consists of acharan sulfate, a GAG with a hybrid structure of heparin and heparan sulfate. All GAG has potency in modulating complement systems[21]. Acharan sulfate can inhibit angiogenesis in an experimental inflammatory model by inhibiting VEGF-induced vascular tube formation[24]. Angiogenesis has a significant role in the pathophysiology of SLE. Increased level of VEGF has been shown to positively correlate with disease activity and systemic organ involvement, especially in lupus nephritis[25].

This study's results did not show the significant effect

of snail mucus (*Achatina fulica)* on the chronicity index, with a p-value of 0.195. However, all the treatment groups showed a lower chronicity index than the positive control group.

The chronicity index of renal histology is associated with whether complete remission is achieved after receiving immunosuppressive therapy for one year, thus predicting the long-term prognosis[26]. In this study, treatments were only given for two weeks. Therefore, it might be the reason why the result was not significant. Additionally, another study stated that kidney function disorders might be predicted by the chronicity index assessed in a repetitive biopsy but not with the first biopsy[26]. Another limitation of this study is that it did not consider the side effects and toxicity, so the safety is still being questioned. However, this research is in line with various other studies to find new drugs for diseases that are difficult to cure [27-29].

Conclusions

Based on the research results, snail mucin *Achatina fulica* significantly reduced the activity index of renal histology in the pristane-induced lupus nephritis mice model but not with the chronicity index. Administering 0.5 ml/day of snail mucus resulted in the lowest activity index.

Conflicts Of Interest

There are no conflicts to declare.

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Authors' contribution

Nurhasan Agung Prabowo, Arief Nurudhin, and Yulyani Werdiningsih are the researcher and data analysts and all of us prepare the manuscript

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