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Research Article

# THE SENSITIVITY TEST OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATES FROM SUSPECT TUBERCULOSIS PATIENTS TO THE SEROMUCOUS OF SNAIL AND CHITOSAN AS AN ALTERNATIVE ANTI-TUBERCULOSIS DRUGS

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**ABSTRACT**

**Objective:** The purpose of this research is to study the sensitivity of *Mycobacterium tuberculosis* (MTB) isolates from suspect TB patients to seromucous of snail and chitosan as an alternative to anti-TB drugs.

**Methods:** The research methods include management specimen, freeze-drying of snail seromucous; formulation of dosage preparation; identification of MTB isolates; and sensitivity testing of MTB isolates to snail seromucous, chitosan, and streptomycin, isoniazid, rifampicin, and ethambutol (SIRE).

**Results:** The characteristics of respondents by sex and age are the majority of male respondents and productive adult age that is 26 years–52 years. MTB isolates used in the study were obtained from the results of the screening of sputum samples of suspect TB patients through microscopic smear examination and molecular rapid test using GeneXpert tools. MTB isolates in patients suspect TB are resistant against seromucous of snails and chitosan that it is compared with SIRE. The dosage of snail seromucous is 8000 mg/l, chitosan 2% is 800 mg/l, and SIRE, respectively (rifampicin 8000 mg/l, isoniazid 20 mg/l, ethambutol 200 mg/l, streptomycin 800 mg/l).

**Conclusion:** MTB isolates from patients suspect who TB is resistant to seromucous of snail (8000 mg/l) and chitosan (800 mg/l).

**Keywords:** Sensitivity, Isolates, *Mycobacterium tuberculosis*, Snail seromucous, Chitosan, Streptomycin, Isoniazid, Rifampicin, Ethambutol.

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## INTRODUCTION

Tuberculosis (TB) is an infection caused by *Mycobacterium tuberculosis* (MTB) and transmission through droplets of sputum sufferers or suspect TB in the air. Organs that are infected are generally the lungs so that it is called pulmonary TB, but it can also attack other organs, namely, lymph, brain membranes, skin, bones, joints, intestines, and kidneys, so it is called extrapulmonary disease. TB can be identified through suspected TB, including coughing continuously for 2–3 weeks or more accompanied by blood, shortness of breath, weak body, decreased appetite, weight loss, night sweats even without activity, and fever for more than a month.

TB treatment lasts long enough that is at least 6 months of treatment which has an impact on the emergence of germ resistance and TB treatment does not work because patients drop out of treatment or take medication irregularly so that multi drugs resistance tuberculosis (MDR-Tb) occurs. Most MDR-Tb occurs due to lack of adherence in the treatment of T. The resistance that occurs can be in the form of primary resistance and secondary resistance. Early detection of MDR-Tb and starting therapy as early as possible is an important factor for achieving therapeutic success.

TB can be cured by administering appropriate anti-tuberculosis drugs (ATD). First-line treatment for TB usually uses isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EB) or streptomycin (SM) as the main choice. There are side effects in MDR-TB therapy and the correlation between cure rates and resistance to anti-tuberculosis medication so that a psychological, social management approach is needed in MDR-TB patients and the presence of a bacterial profile related to resistance against antibiotics against TB treatment.

Snails (*Achatina fulica* Ferussac) contain bioactive compounds found in seromucous or hemolymph. Seromucous of snails have bioactivity as HeLa antitumor and are non-toxic to lymphocyte cells, it can even stimulate lymphocyte proliferation. Snails seromucous as glycoprotein containing carbohydrate; fraction α-1 globulin oromucoid; glycans, peptides, glycopeptides, and chondroitin sulfate. The snail chondroitin sulfate can function as immunomodulation and immunosuppressant [1]. Another substance of snails seromucous is content of glycosaminoglycans, heparin, heparin sulfate, sulfate dermal, and hyaluronic acid; can function as stabilizer cofactors and/or coreceptor for growth factors, cytokines, and chemokines; enzyme activity regulator; molecular labeling in response to cellular damage in the process of wound healing, infection, tumorigenesis; target for bacterial, viral, and parasitic virulence factors; as well as the immune system [2]. Achasin protein in the snail has important biological functions, among others, as a bacterial binding protein (enzyme) receptor [3]. The concentration snail mucus 100% and 5% snail slime cream have an effective effect on accelerating the duration of healing of second-degree (A) burns [4].

Chitosan is a polysaccharide that is found in abundant quantities in nature, especially in waste shrimp shells, crab skin. Chitosan is β-(1.4)-2 amino-2 deoxy D-glucopyranose as a product of chitin deacetylation. The uniqueness of chitosan is polycationic so that it can suppress the growth rate of diarrheagenic *Escherichia coli in vitro* [5]. Synbiotic (Chitooligosaccharide and *Lactobacillus acidophilus*) bio preparation in yogurt can reduce cholesterol levels *in vitro* and *in vivo* [6,7]. Chitosan has been widely used in biomedical and pharmaceutical fields because it is biodegradable, non-toxic, non-immunogenic, and biocompatible with animal body tissues [8]. The combination of 100% snail mucous

**Table 2: Resistance of *Mycobacterium tuberculosis* isolates against seromucous of snail, chitosan, and SIRE**

| No. | Sample | Dilution | Negative control | Chitosan | Seromucoid of snail | Streptomycin (S) | Isoniazid (I) | Rifampicin (R) | Ethambutol (E) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 122 | $10^{-3}$ | 2+ | 2+ | 2+ | 2+ | Negative | Negative | 2+ |
| | | $10^{-5}$ | 1+ | 1+ | 1+ | 1+ | Negative | Negative | 1+ |
| 2 | 172 | $10^{-3}$ | 1+ | 4+ | 4+ | 1+ | Negative | Negative | Negative |
| | | $10^{-5}$ | 15 colonies | 3+ | 3+ | 5 colonies | Negative | Negative | Negative |
| 3 | 197 | $10^{-3}$ | 2+ | 3+ | 2+ | Negatif | Negative | Negative | 2+ |
| | | $10^{-5}$ | 1+ | 2+ | 1+ | Negatif | Negative | Negative | 1+ |
| 4 | 200 | $10^{-3}$ | 4+ | 4+ | 4+ | 4+ | Negative | Negative | 4+ |
| | | $10^{-5}$ | 3+ | 3+ | 3+ | 3+ | Negative | Negative | 3+ |
| 5 | 218 | $10^{-3}$ | 2+ | 3+ | 3+ | 2+ | Negative | Negative | 2+ |
| | | $10^{-5}$ | 1+ | 2+ | 2+ | 1+ | Negative | Negative | 1+ |
| % Resistance | | | 100 | 100 | 100 | 80 | 0 | 0 | 80 |

the proliferation phase of wound healing. The seromucous of snail preparations that have been used in this study as freeze-dried preparation because based on the results of research that is being done shows that snail seromucous freeze-dried has been significant activity on lymphocyte proliferation *in vitro* compared to snail seromucous without freeze-dried. The results of previous studies showed that snail seromucous concentration of 100% is antibacterial against *S. aureus, Candida albicans*, and *Pseudomonas aeruginosa* [18]. The difference in the variation of antibacterial agents because it is influenced by inoculum strains related to the level of resistance of microorganisms as well as the type of antibacterial Achasin protein of genetic expression each snail strain varies. Antibacterial and antifungal test results of meat protein extract from seven different types of snail with diffusion and dilution methods showed that varied minimal inhibition concentration and influenced by snail's ecological conditions [19]. Various types of proteins or known as Achasin proteins in snails have important biological functions, including as a protein-binding receptor (enzyme) for bacteria. Snail mucous is able to be as antibacterial against *Streptococcus mutans* and *E. coli* and inhibits the growth of methicillin-resistant *S. aureus* [20]. The concentration of 100% snail mucus is bacteriostatic against the growth of *S. aureus* and *Salmonella typhosa* [21]. The effectiveness of bactericidal and bacteriostatic snail mucus varies with isolates *Staphylococcus* Sp., *Streptococcus* Sp., and *Pseudomonas* Sp. [22]. A number of protein lectins are known to be contained in snails, namely selectin, galectin, C-type lectins, and fibrinogen-related proteins secreted by snails after an infection which has a role in the process of pathogen agglutination [23]. The results of the characterization of snail protein seromucous profile SDS-PAGE method showed that there were three subunits of protein, namely the range of 55-72 kDa as the Achasin sulfate group that played an antimicrobial role and 1 specific protein subunit 43 kDa as an adhesive protein which was still further investigated [24].

Chitosan synthesis uses samples of shrimp shell or crab shells through the process of deacetylation with 60% NaOH at 60–100°C; deproteinization with 3.5% NaOH, decalcification with HCl 2N, and color removal with acetone and 2% NaOCl [25]. Chitosan has biocompatible, biodegradable, non-toxic, antimicrobial, and hydrating agent properties. Chitosan also influences the process of blood clotting so that it can be used as a hemostatic and positive effect on wound healing.

The content of bioactive compounds in snail seromucous and chitosan can stimulate the function of cellular immunity, namely lymphocyte proliferation and the production of reactive oxygen intermediated macrophages. Seromucoid freeze-drying and chitosan preparations showed significant activity on lymphocyte proliferation *in vitro* compared to snail slime without freeze-drying. Chitosan 5% gave the most effective result toward lymphocyte proliferation activity compared to 100% snail slime and 5% snail slime cream. The snail mucus cream 5% provides a higher proliferative activity than the 100% snail mucus. The mixture of chitosan 5% and snail slime 100%; snail slime 5% cream showed optimum effectiveness against lymphocyte proliferation *in vitro* [26].

The effectiveness of a bactericidal or bacteriostatic drug against MTB isolates can be influenced by the physiological bacterial cells as genetic factors related to the level of resistance or cell virulence and mutation process caused by mutagenic agents in physical chemistry from environmental factors. MTB cells have mycolic acid (trehalose dimycolate) which plays an important role in the interaction of pathogens with the host. Mycolic acid functions were equivalent to lipopolysaccharides in Gram-negative bacterial cells. Mycolic acid influences the function of macrophages which inhibits the fusion of macrophages in host cells against pathogens. The presence of mycolic acid content in MTB plays an important role in the level of resistance of germs to host immune cells and drugs.

The immune response also plays an important role in MTB infection. The immune system plays an effective role in most infections. The risk of developing TB increases if there are conditions that interfere with the immune system such as coinfection with HIV. Macrophages cells play an important role in the immune system host by phagocytosis of cellular antigens. Bacteria in the lungs will be phagocytosed by alveolar macrophages, but MTB found in macrophages can change the acidity of the environment so that the phagosome maturation process stops. This results in a phagosome not being able to fuse with lysosomes and MTB cannot be destroyed and subsequently replicates in macrophages. MTB cells secrete virulence factors such as ESAT-6, CFP-10, and MPT-64. MTB cells have many protein antigens, some of which are in the cytoplasm, cell walls, and others are secreted. Proteins secreted into the extracellular environment by MTB, namely ESAT-6, CFP-10, MPB-70, MPT-64, MPT-63, and MPT-80 can cause an immune response and have diagnostic values [27].

Treatment of TB with ATD so far has been given the right ATD; however, lately, there are many strains of MTB resistant to two or more ATD known as MDR-MTB strains. The prevalence of MDR-TB and extensively drug-resistant TB is higher in the case of recurrent TB treatment compared to the initial TB case and the variation in the level of TB germ resistance to ATD is influenced by age, sex, and region [28]. There are side effects in MDR-TB therapy and the correlation between cure rates and resistance to ATD so that a psychological, social management approach is needed in MDR-TB patients and the presence of a bacterial profile related to resistance against antibiotics against TB treatment. This is due to the patient's ignorance of the disease, poor patient compliance, administration of monotherapy or ineffective drug regimens, inadequate doses, poor instructions, low medication regularity, poor patient motivation, irregular drug supply, poor bioavailability, and poor quality the drug contributes to the occurrence of secondary drug resistance.

The characteristics of respondents by sex and age are the majority of male respondents and productive adult age that is 26 years–52 years. Respondents generally suspect TB is more common in men because the occurrence of cases of TB infection is closely related to the respondent's internal factors or physiological factors, namely genetic, nutritional, stressor, respondent behavior, and external factors, namely