
The Influence of Bioinsecticide Granules of Srikaya (*A. Squamosa*) and Betel Leaves (*P. Beetle*) Extracts on Kidney Histopathology in Mice (*Mus musculus*)

Wike Rosalini¹(corresponding author), Sri Hernawati², Dwi Wahyuni³¹Postgraduate Program of Public Health Science, Universitas Jember, Indonesia; wikerosalini26@gmail.com²Faculty of Dentistry, Universitas Jember, Indonesia; srihernawati.drg5@yahoo.com³Faculty of Mathematics and Natural Sciences, Universitas Jember, Indonesia;
dwiwahyuniwik@gmail.com

Submitted: December 28, 2019 -Revised: January 21, 2020 -Accepted: January 26, 2020 -Published: January 31, 2020

ABSTRACT

The use of chemical insecticides in the long term and high frequency will suppress insects (mosquitoes) and vectors to be tolerant until resistant. Reports of resistance from various species of mosquitoes found in multiple countries even occur in Indonesia in Surabaya. This study aimed to analyze the effect of granules bioinsecticides on the mixture of srikaya seeds (*A. squamosa*) and betel leaf (*P. beetle*) on the histopathology of mice (*M. musculus*) using true-experimental (the post-test only group design). Sample taken by simple random sampling and grouped into each control group and the treatment group totaling 25 mice. Data were analyzed using simple linear regression test. The results showed an overall p-value >0.05, meaning that there was no bioinsecticide effect of a mixture of betel leaf granules (*Piper beetle*) and srikaya seeds (*Annona squamosa*) on the histopathology of the kidney of mice (*Mus musculus*). Required safety testing of target animals in addition to mice and a preliminary examination is needed before the research is conducted so that the results are valid.

Keywords: bioinsecticide; histopatology; *Mus musculus*

INTRODUCTION

The use of chemical insecticides in the long term with high frequency and gradually will suppress and select insect (mosquito) vectors to be tolerant until resistant to it⁽¹⁾. The commonly used insecticide for controlling DHF vectors in both the larval and adult mosquito phases is abate or temephos. Some things that need to be observed are the emergence of resistance from various types of mosquitoes that live in tropical regions such as Indonesia. The opposition has been reported in multiple parts of the world, such as Brazil, Bolivia, Argentina, Cuba, the Caribbean, and Thailand⁽²⁾. In Indonesia, especially in Surabaya, the *Aedes aegypti* larvae reported resistance to abate⁽³⁾. Problems that are complex to tolerant to resistant to mosquito larvae and even mosquitoes. 1985 WHO (World Health Organization) recommends finding breakthroughs and replacing chemical insecticides with biological control or environmental control, one of which is the use of natural substances derived from plants or bioinsecticides⁽⁴⁾.

Srikaya seeds (*Annona squamosa*) is one part of one of the plants that grow in Indonesia. Srikaya seeds are one part of the fruit that has the potential as a natural insecticide. Srikaya fruit containing active compounds such as annonacin and squamosin and simicin⁽⁵⁾. Annonaceous acetogenin contained in srikaya seeds can inhibit ATP production by disrupting the mitochondrial complex I⁽⁶⁾. Besides srikaya betel leaves also contain natural chemicals in the form of phenols and several derivative compounds such as chavicol and eugenol, and contain saponins, alkaloids, tannins, flavonoids and essential oils which can be used as bioinsecticides⁽⁴⁾.

Natural bioinsecticides in the form of granules have the advantage of being more stable and efficient if stored longer if applied to a community and society⁽⁷⁾. A mixture of srikaya seeds and betel leaves is sought to be used as a substitute for chemical insecticides and new bioinsecticide alternatives. In this study, using experimental animals, namely mice (*Mus musculus*) because mice have organs that are almost the same as humans, besides mice are still widely used because they are cheap, fast breed and short birth intervals. The examination carried out on mice as experimental animals is histopathological examination as a reference to identify the damage to one of the organs studied, namely the kidneys. The purpose of this research to analyze the effect of bioinsecticide on a mixture of betel leaf (*Piper beetle*) and cricket seed (*Annona squamosa*) on histopathology of mice (*Mus musculus*).

METHODS

This study used a true-experimental method (the post-test only with control group design), used 4 different doses and each dose was given to 5 mice, the dose used in the study was 1.4 mg/kg-body weight, 2.8mg/kg-body weight, 5.6 mg/kg-body weight and 11 mg/kg-body weight. In the treatment group used four doses and

one control group each so that the number of mice used was 25 mice by considering criteria such as healthy, male sex, age <7 weeks and body weight between 20-30 grams. Mice (*Mus musculus*) were adapted in a laboratory setting for one week. Maintenance was carried out by providing food in the form of pellets from the laboratory and ad libitum drinking water. Mice (*Mus musculus*) were weighed every day and observing their behavior. Mice were declared healthy and could be used for research if their behavior shows no symptoms of pain. Mice (*Mus musculus*) were adapted for seven days in a cage according to their development in the animal laboratory at the Faculty of Dentistry, Universitas Jember. Granule extract of a mixture of betel leaf extract (*P. battle*) and srikaya seed (*A. squamosa*) was given to mice by a predetermined dose at a volume that did not exceed intragastric mice (1 ml). The extract was given seven days every day as much as 0.5 ml in 4 different doses, and each dose was repeated in 5 different mice (*Mus musculus*). The amount of dose given was calculated from mg/kg body weight of mice (*Mus musculus*). On the 7th day, all mice were 12 hours fasted, were not given food and drink. The mice were dissected and their kidney organs taken to make preparations by the procesed fiksasi, dehydration, clearing embedding, sectioning, staining and mounting.

The 5 days after make a histopatology preparation, the rat kidney (*Mus musculus*) was observed under a microscope with 400 x magnification. The results of administration of a mixture of extra granules of Betel Leaves (*P. batle*) and Srikaya Seeds (*A. squamosa*) to histopathology of mice (*Mus musculus*) were analyzed using ordinal logistic regression test.

RESULTS

Histopatology of Mice (*Mus musculus*)

The results of the analysis of the effect of bioinsecticide granules of srikaya seed extract (*Annona squamosa*) and betel leaf (*Piper beetle*) on the histopathology of mice (*Mus Musculus*) with four doses in the treatment and control groups were as follows:

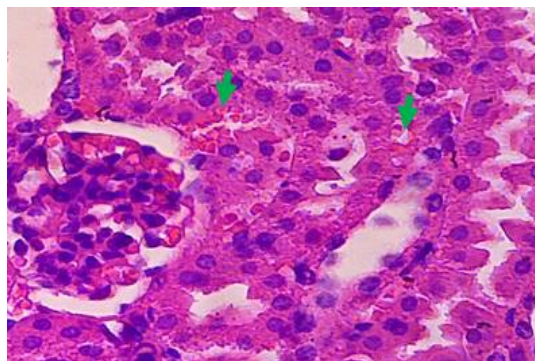


Figure 1. Kidney histopathology of mice (*Mus musculus*) after administration of a mixture of betel leaf extracts and srikaya seed observed with a 400 x magnification microscope. Treatment 1 dose of 1.4 mg/kg, renal histopathology is damaged or necrotic

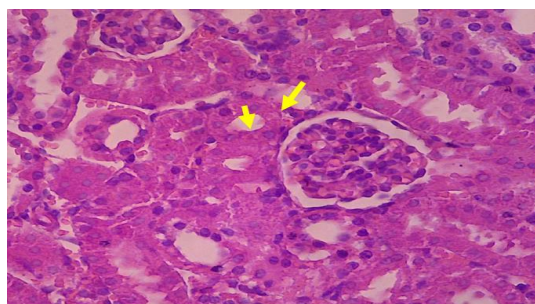


Figure 2. Kidney histopathology of mice (*Mus musculus*) after administration of a mixture of betel leaf extracts and srikaya seeds observed with a 400 x magnification microscope. Treatment 1 dose of 1.4 mg/kg, renal histopathology is damaged or necrotic.

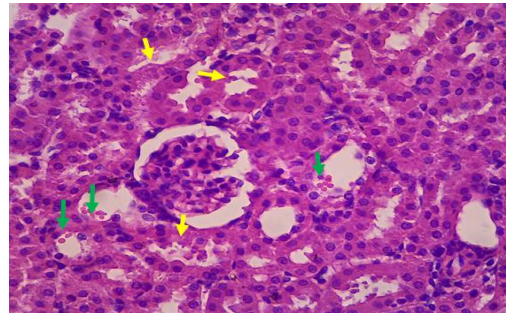


Figure 3. Histopathology of kidney Mice (*Mus musculus*) after administration of a mixture of betel leaf extract and srikaya seeds was observed with a 400x magnification microscope. Treatment 2 at a dose of 2.8 mg/kg body weight, renal histopathology experiences cell necrosis or damage marked by (yellow arrows) and kidney organs experience hemorrhage marked by (green arrows)

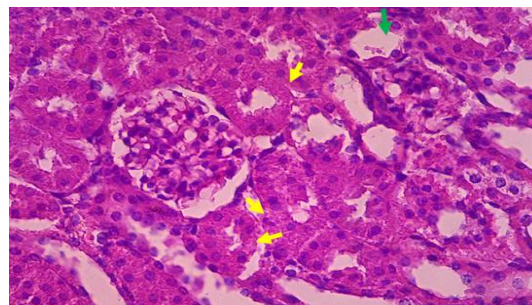


Figure 4. Kidney histopathology of mice (*Mus musculus*) after administration of a mixture of betel leaf extracts and sugar apple seeds observed with a 400x magnification microscope. Treatment 3 with a dose of 5.6 mg/kg body weight, kidney histopathology experiences cell necrosis or damage marked by (yellow arrows) and kidney organs experience hemorrhage marked by (green arrows)

Table 1. The Result of logistic regression test

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 ^a	bioinsecticide			0.000	4	1.000	
	bioinsecticide (1)	-21.608	1.797E4	0.000	1	0.999	0.000
	bioinsecticide (2)	0.000	2.542E4	0.000	1	1.000	1.000
	bioinsecticide (3)	0.000	2.542E4	0.000	1	1.000	1.000
	bioinsecticide (4)	0.000	2.542E4	0.000	1	1.000	1.000
	Constant	21.203	1.797E4	0.000	1	0.999	1.615E9
a. Variable(s) entered on step 1: bioinsecticide							

DISCUSSION

A mixture of seed granules (*Piper beetle*) and srikaya seeds (*Annona squamosa*) were small granules formed that dissolve in water and made the water used not turbid. Bioinsecticide mixture granules that enter the body; one of its functions is to maintain blood composition by preventing the accumulation of toxins and controlling fluid balance in the body. A kidney is an organ that is vulnerable to the occurrence of toxicity, assessment, and consideration in tissue research that is suspected to be disturbed can be observed with histopathological observation in the kidney.

Renal histopathological observations in the control group found necrosis and hemorrhage damage to kidney tissue in all repetitions, whereas histopathological observations in the treatment groups given I, II, III, and IV found damage or changes such as the presence of hemorrhage cells and necrosis. Necrosis is characterized by loose or loss of tissue structure, and cells in kidney tissue experience cell damage ⁽⁸⁾. Damage that occurs can be observed from the part of the kidney that is damaged, namely the tubular cells of the consortium. Damage to

cells in kidney tissue is thought to arise due to several factors one of which is lack of oxygen, extreme temperature changes and or loss due to objects with specific contents⁽⁹⁾, besides that necrosis is also caused by viruses such as distemper virus⁽¹⁰⁾. Damage to the kidneys studied was classified as mild necrosis and was still within safe limits. The treatment group of granules mixed with srikaya seed extract (*Annona squamosa*) and betel leaf (*Piper betle*) had no significant effect on mice (*Mus musculus*).

The researchers believe that the presence of necrosis and hemorrhage in all control and treatment groups had nothing to do with the srikaya seed granules and betel leaves, which were given to experimental animals. Betel plants (*Piper beetle*) contain essential oils of 4.2%, but the main component consists of betlephenol and some deviates, including eugenol allypyrocatechine. Bactericidal properties that have a way of working by increasing the permeability of bacterial membranes, namely eugenol compounds, eugenol bactericidal effect five times that of other phenol⁽¹¹⁾. Natural chemicals such as phenols and their derivatives such as chavicol and eugenol, and contain saponins, alkaloids, tannins, flavonoids, and essential oils can be used as bioinsecticides⁽⁴⁾.

In the research, srikaya seeds were also used as a mixture of betel plant granules containing acetogenin, which gave effects such as hemorrhage and even necrosis⁽⁵⁾. Annonain and squamosin can inhibit ATP synthesis in the mitochondria⁽¹²⁾. Other research shows that annonacin and squamosin act as poisons used as larvicides and mosquitoes as target animals⁽⁵⁾. Research on squamosin and annonacin has been investigated at a more molecular level with cytotoxic effects affecting cell death in living things, annonacin and squamosin are able to inhibit electron transfer at a site I by blocking NSDH enzyme bonds with ubiquinone in the removal of electron cell respiration which consequently inhibits metabolic energy formation⁽¹³⁾. Besides Annonain and squamosin, pomegranate extract is also used as an antibacterial, anti-virus, anti-cancer and anti-inflation all of the pomegranate extract contains several active ingredients that have benefits that are sold together synergistically⁽¹⁴⁾.

CONCLUSION

Based on the results of research conducted by researchers, it can be concluded that Histopathologically, there was no effect of bioinsecticides in the mixture of betel leaf (*Piper beetle*) and srikaya seed (*Annona squamosa*) on the histopathology of the kidney of mice (*Mus musculus*) after giving granules a mixture of betel leaf extract and Piperaya seeds. Further test is needed on other animals or target animals such as mosquito larvae so that the effectiveness of the granules mixed with srikaya seeds and betel leaves can be used for renewal.

REFERENCES

1. George WW. Modes of action for insecticides. Pepticides: Theory and Application. The British Crop Protection. 1983;145-148.
2. Felix. When the larva and adult mosquito are immune to insecticides. FARMACIA. 2008;7(7).
3. Raharjo B. Vulnerability test (Susceptibility) Mosquitoe aedes aegypti (Linnaeus) from Surabaya, Palembang and some regions in Bandung Against Larvasida Temephos. Bandung: ITB; 2006.
4. Wahyuni D. Laboratory Test of Toxic Compounds of Betel Leaf Extract. Spirulina. 2013;1(18).
5. Wahyuni DL. Toxicity of Mix Betel Leaf Extract (*Piper betle*) and Srikaya Seed Extract (*Annona Squamosa L*) on Mortality of Aedes Aegypti L Mosquito Larvae. Jember: Pendidikan Biologi Universitas Jember; 2015.
6. Pardhasaradhi. Antitumor Activity of Annona squamosa seed Extract is Trough The Generation of Free Radicals and Induction of Apoptosis. Indian Journal of Biochemistry and Biophysics. 2004;41(4);167-72.
7. Lathifa. Effect of srikaya Seeds Extract Granules on Morphology of Renal Histopatology. Repository. Jember: Universitas Jember; 2018.
8. Setyowati A, Hidayati D, Awik PDN, Abdulgani N. Histopatology Study of Belanak (*M. cephalus*) Liver in Muara Sungai Aloo Sidoarjo. Surabaya: Program Studi Biologi, Fakultas Matematika Ilmu Pengetahuan Alam, Institut Teknologi Sepuluh Nopember Surabaya; 2010.
9. Berata IK, Winaya IBO, Adi AAAM, Adnyana IBW. General Veterinary Pathology (Patologi Veteriner General). Denpasar: Swasta Nulus; 2011.
10. Fadilah MF, Berata IK, Kardena IM. Histopathological study of dogs with distemper patients is associated with inflammatory cells in the brain and lungs. Bul. Vet. Udayana. 2015;7(2).
11. Inayatullah. The Extra of Green Betel Leaf (*Piper betle L.*) Against Staphylococcus aureus Bacteria Growth. Jakarta: Pendidikan Dokter, FKIK UIN; 2012.
12. Wardhana A, Husein A, Manurung J. Effectiveness of Srikaya Seed Extract (*Annona squamosa L*) with Water, Methanol and Hexane Solvent on Mortality of Boophilus micrplus tick Larvae In Vitro. Bogor: Balai Penelitian Veteriner. 2005;10(2).
13. Londershausen M, Leicht W, Lieb F, Moeschler H, Weiss H. Annonins mode of action of acetogenin isolated from annona squamosa. 1991.
14. Hernawati S, Rantam F, Sudiana K, Rahayu R. The Pomegranate Extract (*Punica granatum L*) Terhadap Ekspresi Wild p53 pada Sel Ganas Rongga Mulut Mencit Strain Wiss Webster. Dental Jurnal. 2013;46(30).