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Safety of Bioinsecticide Ekstract Sugar Apple Seed's Granule (Annona squamosa L.) on Histology of White Rat (Rattus norvegicus B.)

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Abstract: ***The granules of sugar apple seeds extract (Annona squamosa L.) have been taken as the natural larvacide an economical in eradicating mosquito flicks of Aedes aegypti L. because of its the content of toxic compounds such as acetogenin, alkaloids, and flavonoids. In its use it is feared to cause toxic in the environment, especially the class of mammals as non-target animals, especially kidney organ. The kidney is an organ that is susceptible to toxic effects, since it receives 25-30% of the blood circulation to be cleaned, so that as a filtration organ there is a possibility of pathological changes. This study aims to determine the effect granules of sugar apple seeds extract (Annona squamosa L.) on the histology of white rat (Rattus norvegicus B.). Kidney histology observed as fat degeneration, hydrophic degeneration, and necrosis of tubular cells occurred in one field of view. The concentration given was 1 mg, 2 mg, 4 mg, and 8 mg while the negative control induced 2 ml aquades and positive control using abate 100 mg for 14 days with sonde method. The analysis used in this research is descriptive analysis with scoring. The results showed that granules of sugar apple seeds extract did not give effect to histology of white rat (Rattus norvegicus B.).***

Index Terms: Granules Extract, Safety Bioinsecticide, Histology, Sugar Apple Seed's Granule

I. INTRODUCTION

The sugar apple plant (Annona squamosa L.) is now scattered throughout Indonesia, although it is still limited to planting. People only use fruits for consumption [1]. The seed of sugar apple is one part of the sugar apple plant (Annona squamosa L.) that secretes into a natural insecticide. The seeds of contain chemical compounds of acetogenin consisting of annonain, squamosin, and asimisin [2].

Sugar apple seeds are oval, shiny and smooth, black or dark brown, and measuring 1.3-1.6 cm with a weight of 5-18 grams [3]. The seeds of srikaya also contain alkaloid compounds that can cause insect death through the mechanism of contact toxins and stomach toxins. In addition, alkaloids are thought to have the effect of inhibiting lipase enzyme activity because it has a chemical structure similar to orlistat (the presence of N elements). While flavonoids are respiratory inhibitors by working to damage the siphon and weaken the nervous system by inhibiting the action of acetylcholinesterase enzyme [4]. Efforts to control insects have long been done and almost all people use synthetic insecticides. Abate is one of the synthetic insecticides that are often used in the community to eradicate mosquito larvae. Increased use of synthetic insecticides results in resistance to targeted insects, as well as residues that can pollute the environment [5]. Based on these facts need further action with security testing on mammals to be made as bioinsektisida that is safe for the surrounding environment, especially in mammals.

In addition to using extracts, the seed granules of srikaya seed (Annona squamosa L.) has now become an economical natural insecticide in the control of Aedes aegypti L larvae. The use of natural insecticides in granule form is more stable if stored in a long period of time and more applicable in its use by the community [6].

Srikaya plants (*Annona squamosa* L.) can grow on all soil types with pH 6-6.5. Thus, cultivated srikaya planted in a rather acidic environment. The most preferred type of soil is the soil containing lots of sand and lime. The area with an altitude of 100-1000 m above sea level is the height favored by srikaya plants, but the best height is at an altitude of 100-300 m above sea level. While the preferred temperature of srikaya plants ranges from 20-25^{0}C [1].

The seed of srikaya (*Annona squamosa* L.) is one part of the srikaya plant (*Annona squamosa* L.) that has the potential to be developed as bioinsecticide. As in the research which states that the seeds of srikaya can be used to kill lice and insects. The seeds of srikaya consist of various toxic compounds such as acetogenin, alkaloids, and flavonoids [7]. The toxicity possessed by this plant has a level of toxic residue that is easily lost which is about 48 hours after use [8]. The compound of acetogenin in srikaya seed consists of annonain which has chemical formula C35H64O7 and squamosin with chemical formula C37H66O7. These compounds have properties as insecticides, acaricides, antiparasites and bactericides with inhibitor mechanism [9].

Alkaloids in somatic seeds can act as insecticides, larvacides, insect repellents and inhibitors [10]. Alkaloids are secondary metabolites that are plant antioxidants and are capable of causing insect death through toxic mechanisms of contact and abdominal toxins and are easily broken down if stored for long periods [11].

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Alkaloid compounds are suspected to have the effect of inhibiting lipase enzyme
activity because it has a chemical structure similar to orlistat (the presence of N elements). While flavonoids are respiratory inhibitors by destructive siphon work and weaken the nervous system by inhibiting the action of enzyme acetylkolinesterase [12]. Granules are clumps of small particles that can be stored for a long time, and are more stable in their use. Granules have low concentrations of active ingredients and varying sizes. The size of the granules usually ranges from 4-12 mesh sieve,
but the granular size can be adjusted according to the research needs [13]. Granules are products produced by the granulation process which will then be used as solid dosage. Method of making granules there are 2 types, namely wet granulation and dry granulation [14].

Research conducted found that the granule of extracts of seeds srikaya (*Annona squamosa* L.) can kill 50% larvae of Aedes aegypti L. mosquitoes of 1.08 ppm within 24 hours [15]. Biopesticides are usually biodegradable, so they do not pollute the environment and are relatively safe for humans and livestock because the residue is quickly lost. However, it is possible that organic compounds present in biopesticides can affect organs in non-target animals [16]. The use of herbs as traditional medicine for long periods of time can cause symptoms of toxicity. Any drug taken orally will undergo a process of absorption, metabolism and excretion. The kidneys have an important function in the process of excretion of ingredients that enter the body orally.

The kidney is an organ that is susceptible to toxic effects, since it receives 25-30% of the blood circulation to be cleaned, so that as a filtration organ there is a possibility of pathological changes. Therefore, there is a need for further research on the effects that will be generated by the extract of the seed granules srikaya (*Annona squamosa* L.) against the kidneys of white rats (*Rattus norvegicus* B.).

As a result of the use of natural larvacide is seen from the histology of the kidneys that occur in non target animals. This study uses test animals that can be synonymous with events that may arise when the granules are used by humans. Humans are a class of mammals so that researchers use a test animal that is white mouse (*Rattus norvegicus* B.). these wistar white rat strains are included in the mammalian class to allow for the same effects as humans. The observed histologies are hydrophic degeneration, fat degeneration, and necrosis.

II. MATERIALS AND METHODS

This research is a type of laboratory experimental research. This series of research was conducted in Biomedical Laboratory of Faculty of Dentistry of Jember University for acclimation, treatment of white rat (Rattus norvegicus B.), treatment to histology preparation, while histology observation was done at Biology Education Laboratory of FKIP University of Jember. The implementation of this study began in January 2018.

The procedures include sterilization of tools and materials, maintenance and treatment of white rats (Rattus norvegicus B.), administration of extracts of somatic seed extract (Annona squamosa L.), surgery, renal histology preparation, histologic observation. The concentration given was 1 mg, 2 mg, 4 mg, and 8 mg while the negative control induced 2 ml aquades and positive control using abate 100 mg for 14 days. The analysis used in this research is descriptive analysis.

III. RESULT AND DISCUSSION

The results of histologic observation of rats of white rat (*Rattus norvegicus* B.) after granula extract of seed srikaya (*Annona squamosa* L.) orally. Figure 1, 2. 3 and 4 represents the renal histology of white rats (*Rattus norvegicus* B.) treatment group, figure 5 is positive control group, and figure 6 is negative control can be seen in the following.

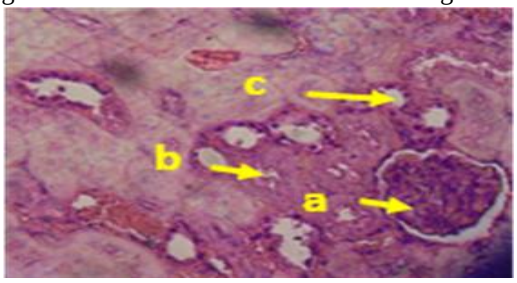

Figure 1. Renal histology of treatment group 1 (P1) with HE staining. a = glomerolus; b = proximal tubules; c = distal tubule (Magnification 400 X)

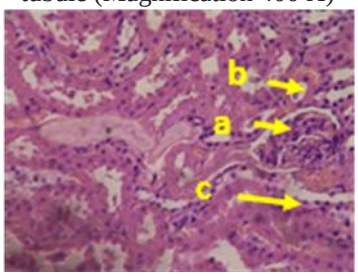

Figure 2. Renal histology of treatment group 2 (P2) with HE staining. a = glomerolus; b = proximal tubules; c = distal tubule (Magnification 400 X)

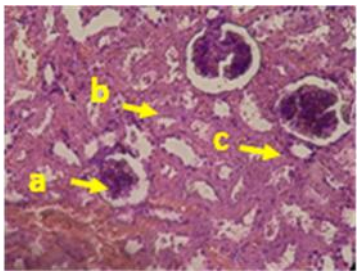

Figure 3. Renal histology of treatment group 3 (P3) with HE staining. a = glomerolus; b = proximal tubules; c = distal tubule (Magnification 400 X)

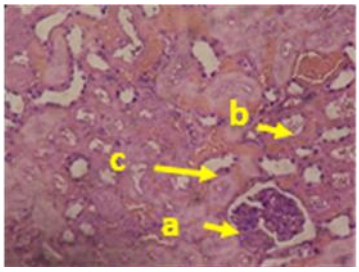

Figure 4. Renal histology of treatment group 4 (P4) with HE staining. a = glomerolus; b = proximal tubules; c = distal tubule (Magnification 400 X)

Figure 5. Renal histology of treatment group (K+) with HE staining. a = glomerolus; b = proximal tubules; c = distal tubule (Magnification 400 X)

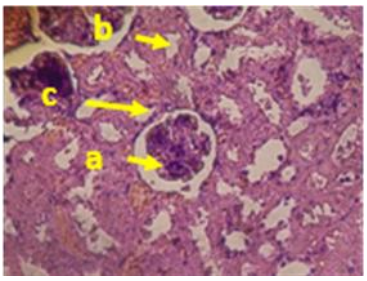

Figure 6. Renal histology of treatment group (K-) with HE staining. a = glomerolus; b = proximal tubules; c = distal tubule (Magnification 400 X)

From this research it can be seen that the histology of kidney rats in the treatment group (figure 1, 2, 3 and 4) and control group figure 5 (K+) and figure 6 (K-) did not show any difference. In this microscopic observation there is no sign of damage such as degeneration of fat which is usually characterized by a yellow vacuole surrounding the tubule epithelial nucleus, a hydrophic degeneration characterized by tubular epithelial tubular epithelium, and necosis characterized by loss of the tubule epithelial nucleus. Thus, from the observation it can be seen that there is no effect of extract of somatic seed extract (*Annona squamosa* L.) on the histology of rats white rat (*Rattus norvegicus* B.) [15].

The mosquito's digestive system is different from mammals. Mammalian digestive system is much more complex than the digestive system in mosquitoes. Mosquitoes have digestion outside the intestinal tract (Elstrainstestinal Digestion) in which mosquitoes will release a special fluid to suck blood without blood clotting. The mosquito has blood on its body that resembles a mammal called hemolymph. Hemolymph is a liquid consisting of water, inorganic salts, proteins and fats. These materials serve to regulate the body temperature of mosquitoes, transporting nutrients, and immune. Blood circulation in mosquitoes is different from mammals. Circulatory system in the mosquito that is an open circulation system, whereas in mammals, especially white mice have a closed circulatory system.

One of the additional ingredients used in the manufacture of granules is lactose. Lactose ($C_{12}H_{22}O_{11}$) can condense granule masses and readily mix homogeneously. Lactose is a form of disaccharide of carbohydrates that can be broken down in the simpler form of galactose and glucose. This glucose will be through the digestive mechanism to the small intestine and absorbed into the bloodstream. This blood will later be filtered in the kidney which is one of the organs of excretion in mammals.

Kidney is the main organ of drug excretion. Through urine, the kidneys excrete ingredients that are no longer needed by the body. Urine is the main path of excretion of toxicants, consequently the kidney has a high volume of blood flow. Kidney is prone to toxic substances, if too many toxic substances in the kidney will result in organ damage. The proximal part of the proximal tubule is the part that has an important role in the reabsorption of glucose and other important substances that are still needed by the body [16]. Proximal tubular epithelial cells are particularly susceptible to toxic substances. This is because in the proximal tubules occurs the process of absorption and active secretion, and levels of cytochrome P450 in the proximal tubulus is higher to activate toxic substances, so often become the main target of toxic substances. Exposure to proximal tubules by toxic substances will continuously lead to cell death or necrosis.
Renal damage the response of kidney damage produced in the direction of the higher concentration of toxic compounds that concern the organs of the kidney. Kidney damage is also associated with the ability of the kidneys to concentrate xenobiotic substances in the cells. Damage to proximal tubules caused by toxic substances is influenced by several factors including the type of chemical compound, dose, and duration of exposure of the compound. Higher concentrations will result in damage to the proximal tubules becoming wider [17].

IV. CONCLUSION

Granules extract of sugar apple seeds (Annona squamosa L.) are safe against the histology of the white rat (Rattus norvegicus B.) because the results are not found in tissues such as hydrophic degeneration, lipid degeneration, and necrosis of tubular epithelial cells.

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