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Research Article

Toxicity Test Pediocin N6 Powder Produced from Isolates Pediococcus Pentosaceus Strain N6 on White Mice

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ABSTRACT

Pediocin N6 powder is a bacteriocin the heat resistant derived from isolates *Pediococcus pentosaceus* strain N6. These were isolated from water source heat Rimbo Panti West Sumatra. Pediocin N6 powder has high antimicrobial activity, so the potential to be used as biopreservatif on meat and food processing industry which involves heating. Toxicity test was conducted to determine the effects of the toxic effect of a single dose of oral Pediocin N6 powder in test animals male white mice to determine the LD_{50} and see changes in body weight of mice for 15 days of treatment. Changes in body weight of mice was determined by using non factorial experiment in a completely randomized design consists of 4 treatments and 5 replications. The test animals were divided into 4 groups and each treatment consisted of 5 mice. The treatments tested consisted of Pediocin N6 powder 5000 mg/kg body weight, 10,000 mg/kg body weight, 15,000 mg /kg and 20,000 mg /kg body weight of mice. The test results showed that the Pediocin N6 powder up to a dose of 20 000 mg/kg in a single oral dose administration, there are no death of mice to 15 days of treatment. Based on the LD_{50} value of a single oral dose can not be calculated, based on it can be stated LD_{50} value pseudo Pediocin N6 powder greater than 20,000 mg /kg in male mice. The average changes in body weight of mice at a dose of Pediocin N6 powder treatment of up to 20,000 mg/kg every 2 days weighing from day 1 to day 15 of 2.1 gr. Based on these tests Pediocin N6 powder safe used as industry biopreservatif on meat and food processing involves heating.

Key word: Toxicity Test, Pediocin N6 Powder, White Mice, Biopreservatif on Meat

1. Introduction

Lethal Dose 50 is a quantity derived statistically, in order to declare a single dose of something expected compound can be deadly or cause significant toxic effects in 50% of experimental animals after treatment. LD_{50} is a quantitative benchmark that is often used to express the lethal dose range (Loomis, 1978).

Toxic effects on living creatures can be seen and can not. When the absorbed dose is relatively small, the damage can be limited to a few cells only. There are still quite a lot of healthy cells to keep normal function of organs. If the cells were damaged, then the organ can no longer function normally. At that time usually poisoning (toxic work) appeared, generally as a disease process that is integral to the individual (Koeman, 1987).

The main objective of acute toxicity test of a drug or chemical is to establish the potential for acute toxicity, the dose range lethal or toxic dose related drugs, at one or more test animals. Moreover, this test is also intended

to assess various clinical symptoms arise, the toxic effects of typical and intermediary mechanisms deaths of test animals. The initial criteria are often used to evaluate the toxicity testing of new compounds commonly used as an index to estimate mortality lethal dose that may occur in humans. The LD_{50} alue is the amount of the dose of a compound that can cause death in 50% of the population in a given period of time (Loomis, 1978).

Pediocin N6 powder including class IIa bacteriocin which is resistant to heat, produced from isolates of *Pediococcus pentosaceus* strain N6 and has high antimicrobial activity mainly against pathogenic bacteria *Listeria monocytogenes.* One of the requirements for the application of an antimicrobial peptide as food preservative would be the evaluation of its immunogenicity and Also the in vitro and in vivo toxicity. The testing of a potential antimicrobial food would Consider repeated and daily administration of the substance for a required time period to access a possible chronic toxicity (Pariza and Foster, 1983 and Pariza and Cook, 2010). The route of administration should be the same proposed for use in humans (Food and Drug Administration, 1988 and Post, 1996) and toxicological studies involving animals are a major component of safety assessment of bacteriocins (Moreno *et al.,* 2000).

Pediocin N6 powders have the potential to be used as a natural preservative in food. According to Tagg *et al*. (1976), bacteriocins as food biopreservatif must meet criteria such as preservatives or food additives among other more secure for consumers, has a bactericidal activity against the Gram-positive and Gram negative, stable, evenly distributed in the food system and economical. Therefore it is necessary for toxicity tests to determine the toxicity of the Pediocin N6 powder through a test dose of a toxic or lethal dose (LD_{50}) in test animals male white mice.

2. Materials and Methods

2.1. Material

Male white mice Swiss types 2- 3 months old as much as 20 fish weighing 20 to 25 grams, feed pellets white mice and Pediocin N6 powder.

2.2. Giving a Dose of Pediocin N6 Powder Solution

Pediocin N6 powder produced from isolates *Pediococcus pentosaceus* strain N6 pure. Production of Pediocin N6 powder capsule do with the material as much as 20% consists of maltodextrin and skim milk at a ratio of 5: 1 (83.3%: 16.67%) according to the methods Kailasapathy (2002) and the Pediocin N6 powder in it as much as 20%. The dose of Pediocin N6 powder solution according to the method Loomis (1987), appropriate treatment dose divided mice body weight plus 5 ml of water. A treatment consisting of 5000 mg / kg, 10,000 mg /kg, 15,000 mg /kg and 20,000 mg /kg, then reconstituted with 5 mL of water. For the treatment of 5000 mg /kg means Pediocin N6 powder given as 125 mg then added 5 ml of water and so on appropriate treatment dose. Award solution Pediocin N6 powder done every morning during treatment. Before administering solution Pediocin N6 powder, mice were fasted for 4 hours before treatment but still were given a drink and 2 hours after treatment.

2.3. Granting Volume Solution Award Pediocin N66 Powder

Pediocin N6 powder solution according to the methods Kailasapathy (2002), given according to the weight of mice treated mice divided heaviest weight multiplied by 1 ml. Award solution Pediocin N6 powder done orally. Treated mice based on weight, volume solution of Pediocin N6 powder given as much as 0.8 g /ml, 0.84 g /ml, 0.88 g /ml, 0.92 g /ml, 0.96 g /ml and 1 g /ml.

2.4. Acute Toxity

Acute toxicity was assessed with 20 male mice and Pediocin N6 powder solution was administered orally at a dose according to treatment by Loomis (1987). After administration, the animals were kept under observation for a minimum of 48 h. The number of animals killed for each of the doses was Noted and the LD_{50} calculated by the Up and Down method, the which is one of the most used to reduce the number of animals used (Botham, 2004). In this study tested the dose of Pediocin N6 powder lowest 5.000 mg /kg and the highest dose is 20,000 mg /kg.

2.5. Experimental Animals

Swiss type male mice as many as 20 birds were used for the experiments. Mice used were weighing between 20 and 25 g were Provided and kept in plastic boxes with food and water ad libitum. The animals were used in experiments after a period of 7 days of adaptation in captivity, with regular 12 h light-dark periods and an ambient temperature of 20°C (National Institutes of Health, 1996). The procedures used in the assays were approved by the Internal Committee of Animal Ethics-UFSM (protocol number 68/2010), and conform to international standards of animal welfare, as specified by the CIOMS International Guiding Principles for Biomedical Research Involving Animals, Geneva, 1985. Pediocin N6 powder solution was administered orally as prescribed treatment, for 15 days of treatment. The feeding is done 2 times a day ie morning and evenings as much as 5 g /head/day. Mice were fasted meal 4 hours before treatment but still were given a drink and 2 hours after treatment. Weighing mice conducted 2 days for 15 days of treatment. Observed number of deaths of test animals for 24 hours after treatment and continued observation is followed up to 15 days. Number of dead white mice data processed by the method of Reed and Muench.

2.6. Calculation of LD50

The number of dead mice was calculated by LD_{50}. LD_{50} data were analyzed by the method of Reed and Muench (1938) is by calculating the proportion of the distance is then determined logarithm dose comparison.

LD_{50} is determined by adding the log dose is low and the product of the distance proportion ratio higher dose to the lowest dose.

The equation to obtain LD_{50} namely:

$g = h \times i$, $y = g + \log s$, $LD_{50} = \text{anti log } y$

Where :

h: pole

A: The percentage of deaths smaller and closest of the 50%

b: a larger percentage of deaths and the closest of the 50%

i: Increase dosage

k: The dose that causes the death of the larger and most close to 50%

s: The dose that causes the death of smaller and closest of the 50%

g: The result of multiplying the increase in dose to the size of the distance

y: The result of the addition between g and logs

2.7. Preference test

The method used is non factorial design in completely randomized design consists of 4 treatments and 5 replications. The test animals were divided into 4 groups and each treatment consisted of 5 mice. The treatments were tested in accordance Loomis (1978), consisting of:

1. Powder bacteriocins as much as 5000mg/kg body weight of mice.
2. Powder bacteriocins as much as 10,000mg/kg body weight of mice.
3. Powder bacteriocins 15,000 mg/kg body weight of mice.
4. Powder bacteriocins as much as 20,000 mg/kg body weight of mice.

Table 1. The Number of Deaths Mice During Treatment

Dosis (mg/kg BB)	Deaths Mice the Sum	The Life Mice
5000	0	5
10.000	0	5
15.000	0	5
20.000	0	5

LD_{50} Calculation of, $g = h \times i$, $y = g + \log s$, $LD_{50} =$ log y Against

Point Based on up: $h = \frac{50\%-a}{b-a}$, $i = \log \frac{k}{s}$

$h = \frac{50\% - 0}{0} = 0$ $i = 0$, $g = h \times i = 0$

$y = g + \log s = 0$, LD_{50}= log y Against, LD_{50} = log 0 Against = 0

Measured Variables

Changes in body weight of mice every two days after a single dose of oral solution Pediocin N6 powder.

3. Results and Discussion

The number of deaths of test animals during 15 days of treatment is oral administration of a single dose of Pediocin N6 powder solution of can be seen in Table 1. The results of the above study showed that giving a solution of Pediocin N6 powder until a dose of 20,000 mg / kg administration of a single oral dose in mice did not reveal any mortality up to 15 days of treatment. Based on the LD_{50} value of a single oral dose can not be calculated, based on the results of this study can only be expressed LD_{50} value pseudo Pediocin N6 powder greater than 20,000 mg/kg in male mice. Based on these tests show that the Pediocin N6 powder not toxic for white mice. This means Pediocin N6 powder safe used as biopreservatif in food because it comes from isolates of Lactic Acid Bacteria (LAB) *Pediococcus pentosaceus* strain N6. According Alakomi *et al.* (2000), this bacterium include microorganisms GRAS (Generally Recognized As Safe) or group of microorganisms that safely be added in food because it is not toxic so it is known as Good Grade Microorganisms are microorganisms that are not a health risk. Furthermore, according to Barefoot and Klaeuhammer (1983), bacteriocins are antimicrobial compounds easily degraded by proteolytic enzymes in the digestive systems of humans and animals. Bacteriocins produced by lactic acid bacteria is very advantageous in the food industry, especially in the fermented food product as the activity could inhibit the growth of some bacteria contaminants cause decay and diseases are transmitted through food (food borne illnes). The addition of bacteriocins in foods other than to prevent spoilage as well as to extend the storage time of food and inhibits the growth of pathogenic bacteria.

According Jeevaratnam *et al*. 2005, the pickling process food combined with heating in industrial processes and reduce the number of pathogenic microbes in this process involves microbes. Microbial resistance in industrial processes is a problem, and therefore in need of food preservation modern technology with a stable microbial warming and improve the quality of the food. Preserving biological microbes involves assistance in the form of BAL. LAB produce lactic acid component causing pathogenic bacteria are not able to grow more food. BAL utilization as a preservative is indispensable because it has antibacterial activity and resistance to heat.

According Bhunia *et al.* (1990) evaluated the immunogenicity of pediocin PA-1 (ACh) to mice and found that it was not immunogenic for animals. Short-term administration of diets containing nisin (Nisaplin) induced an increase of of both CD4 and CD8 T-lymphocyte cell counts and Also a Decrease of B-lymphocyte counts. The macrophage / monocyte fraction was isolated from peripheral blood Became Significantly Increased after long-term administration (100 days) of Nisaplin-containing diets (De Pablo *et al*., 1999).

Although Several antimicrobial peptides have been purified and Characterized there are currently few studies on acute toxicity for comparison. Extensive toxicological studies Showed that nisin intake does not cause toxic effects to the human body with a Reported LD_{50} of 6950 mg / kg, the which is similar to salt, when Administered Orally (Jozala *et al*., 2007). Pediocin PA-1 is

another bacteriocin that has been used for the same purpose, and studies on its toxicity have been Reported (Bhunia *et al*., 1990 and Dabour *et al*., 2009), Although its use was not yet recommended by WHO (Drider *et al.*, 2006). Some authors have associated high LD_{50} of bacteriocins with digestive enzymes capable of Rapidly inactivating Reviews These substances, being trypsin and chymotrypsin produced in the pancreas and released into the small intestine a prime example (Hara *et al*., 1962, Eckner, 1992 and Cleveland *et al* ., 2001, Deegan *et al.,* 2006). In this regard, the peptide P34 is sensitive to trypsin as well (Motta *et al*., 2007a). Claypool *et al.* (1966) evaluated the effect of nisin in milk chocolate consumed Orally and Noted that only ¼ of the original concentration was detected in saliva after a minute of use. Besides this, some bacteriocins can also be sometimes sensitive to ptyalin, being not detected in human saliva 10 min after the consumption of a liquid containing bacteriocin (Chandrapati and O'Sullivan, 1998).

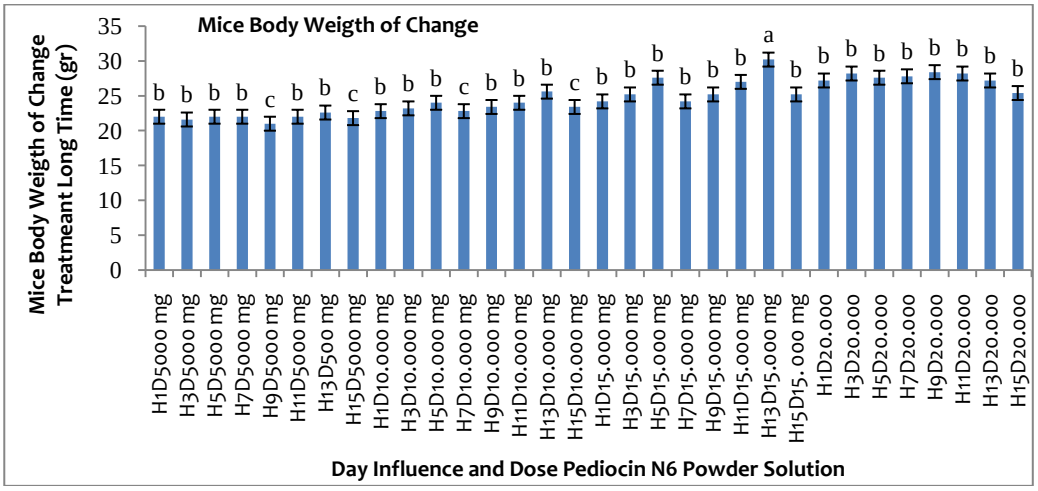

Figure 1. Graph Effect of Single Dose Oral Solution Pediocin N6 Powder Mice Against Weight Changes During Treatment

Developmental changes in body weight of male mice for 15 days treatment of a single dose of an oral solution of Pediocin N6 powders can be seen in Figure 1.The results of the analysis of changes in body weight of mice to a single dose of an oral solution of Pediocin N6 powder on white mice provide a significantly different effect to increase body weight in mice between the 13th day of treatment, the dose of 15,000 mg/Kg BW (H13D15.000 mg/Kg BW) with other treatments. Based on the results of treatment rataannya H13D15.000 mg/kg resulted in changes in body weight were higher at 30.2 grams. The use of solution Pediocin N6 powder to 20,000 mg /kg also showed changes in weight gain in mice. This shows that the administration of a solution of Pediocin N6 powder until a dose of 20,000 mg /kg did not cause toxic or poisonous to the rats on the contrary increase the body weight of mice. This is because the solution Pediocin N6 powder is a protein that is safe to eat mice can even be used as a source of protein for the body of mice and promote weight loss. According to Tagg *et al.* (1976), bacteriocins is a protein compound (such as peptides) that is bactericidal against microbes (bacteria) that if the terms of filogeniknya have close relations with the bacteriocin-producing microbes. According to Salminen *et al*. (2004), bacteriocins is a protein or peptide compounds that can didgradasi by protease enzymes in the digestive tract of humans and animals. This causes the bacteriocins can be used as an antimicrobial agent that is safe to preserve food. Additionally bacteriocins can inhibit bacterial spoilage and pathogenic bacteria in food.

Pediocin N6 powder dosing 15,000 mg /kg on day 1 until day - 12 happens to weight gain in mice but not statistically significant influence. Changes in body weight of mice to give real effect to raise the weight of mice on day 13 and then decreased at day -15. Dosing bacteriocins 15,000 mg /kg give real effect to increase body weight in mice as bacteriocins are proteins that can be used by the body in mice as a source of protein for weight gain in mice. According Savadogo *et al.* (2006), Bacteriocins a protein molecule or peptide ekstrasellular which have bactericidal or bacteriostatic action against bacteria that have a close kinship. The bacteriocins can be degraded by proteases in the digestive tract. Bacteriocins are irrevesibel, easy to digest, positive effect on health and active at low concentrations.

Treatment dosing Pediocin N6 powder to 20,000 mg/kg (H15D20.000 mg/Kg BW) influence changes in weight were not significantly different from other Pediocin N6 powder treatment, this suggests that the intake of nutrients derived from mice to growth-treatment the same treatment. The average weight gain in mice at a dose of treatment Pediocin N6 powder 20,000 mg/kg every 2 days weighing from day 1 to day 15 of treatment of 2.1 gr. Means there is weight gain of 1 g/head/day, corresponding according to Smith and Mangkoewidjojo (1988), that the normal range of the United Nations in mice by 1 g/ head /day. This is because the nutritional content of this treatment according to the needs of mice than that of male mice is more efficient in the use of feed.

4. Conclusion

The results of toxicity testing solution on the Pediocin N6 powder white male mice showed that solution Pediocin N6 powder. Until a dose 20.000 mg /kg in a single oral dose administration did not reveal any mortality up to 15 days of treatment. Based on the LD_{50} value of a single oral dose can not be calculated, based on it can be stated LD_{50} value pseudo Pediocin N6 powder greater than 20,000 mg /kg in mice. This caused Pediocin N6 powder solution is a protein that can be degraded by proteolytic enzymes in the digestive channel animals and humans that are safe for consumption. The average changes in body weight of mice at a dose treatment solution Pediocin N6 powder to 20,000 mg /kg every 2 days weighing from day 1 to day 15 of 2.1 gr. Based on these tests Pediocin N6 powder safe used as indstri biopreservatif on meat and food processing involves heating.

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