International Journal of Health Sciences and Research
Vol.13; Issue: 2; February 2023
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Toxicity Test of Strong Drug Using the BSLT (*Brine Shrimp Lethality Test*) Method

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DOI: https://doi.org/10.52403/ijhsr.20230228

ABSTRACT

One of the methods to test cytotoxic materials is to test the toxicity of Artemia salina leach shrimp larvae (Brine Shrimp Lethality Test). This method is often used for pre-screening the active compounds contained in plant extracts because it is cheap, fast, easy (no need for aseptic conditions), and trustworthy. This study aims to determine the acute toxic effects of the powerful drugs used by the public. This research was experimental research using Post Test Only Control Group Design. It used 120 larvae as test animals which were divided into four groups. Each group contains ten larvae. Each group is done by the replication of research two times. As the test component, the cause of bitter melon is given through the media, which contains larvae as the animal test. The final extract concentration in the media, which contains larvae consecutively as the group of 1, is 4000 ppm, 2000 ppm, and 0 ppm for negative control. The result is against larvae that died 24 hours after the component test was given. Through the data, the LC_{50} value of strong herb was analyzed by probit analysis using Excel for windows. This study shows the load concentration extract in a medium can kill the larvae in a row with a concentration of 4000 ppm and 2000 ppm. The results of probit analysis showed that there is no toxicity to Artemia salina leach larvae. Then it is concluded that there is no potential for acute toxicity according to Artemia salina leach when it was tested using BLST.

Keywords: brine shrimp lethality test, herbal, strong medicine, acute toxicity, Artemia salina leach.

INTRODUCTION

Indonesia is a country that has a lot of diversity potential, both habitat and flora and fauna. This diversity gives Indonesia a lot of biodiversities, especially in herbal plants, which have not been utilized optimally because most of Indonesia's herbal wealth is still not properly explored, so many of these herbal plants cannot be utilized for the development of the traditional medicine industry. The use of herbal plants for traditional medicine has been integrated into the community. It is because herbal plants have several advantages, including easy production, easy to obtain, and affordable prices [1; 2].

Even though modern medicines have dominated formal health services, traditional medicines are still important and continue to grow. [3] Traditional medicine cannot be separated from the lives of most Indonesian people because it is closely related to the nation's culture. According to WHO quality standards, traditional medicines must meet several requirements, including quality, safety, and efficacy. [4] To fulfill these requirements, efforts are needed to ensure the safety of herbal medicines through preclinical tests, including toxicity and activity tests. If these conditions are met, the safety tests of these herbal medicines can continue to the clinical trial stage.

Strong medicine is one of the traditional medicines widely circulated and consumed by the public. Men often use strong drugs to increase sexual stamina or libido. The

composition of strong drugs that are commonly found in traditional medicine (jamu) includes Gingko Biloba Powder and Panax Ginseng (Root).

The use of medicinal raw materials, whether made naturally or synthetically, must be tested for toxicity beforehand so that in an application, it can be declared safe, and it is known how much toxicity is contained in the drug substance. A toxicity test is one of the biological activity tests on extracts or plant isolate fractions by observing the response to death in experimental animals. Experimental animals for toxicity tests usually use fish, mosquito larvae, and shrimp larvae. Mortality of experimental animals is thought to be in response to the effects of certain compounds.

In this study, I used strong drugs as the main ingredient because many types of strong drugs are circulating in the community, both licensed and unlicensed. So this research was aimed at looking at the acute toxic effects of strong drugs that are commonly used by the public, and due to the limitations of the researchers, the researchers used the Brine Shrimp Lethality Test (BSLT) method as a method to see toxic effects because apart from being cheap, easy, and also fast results. Based on the description presented above regarding the strong drugs circulating in the community, this paper will specifically discuss how much LC50 value is contained in the drugs purchased by researchers. This study aimed to determine the acute toxicity potential of strong herbal medicine according to the Brine Shrimp Lethality Test (BSLT) method.

LITERATURE REVIEW

Toxicology is the knowledge of the toxic effects of drugs on the body. In the pharmacodynamics group, the therapeutic effect of drugs is closely related to the toxic effects of drugs or herbs. Toxicology is an older science than pharmacology. [5] The field of toxicology studies the toxic properties of chemical substances on living things and the environment. Humans use at least 50,000 chemicals, and because it is unavoidable, it requires great awareness of the chemicals in drugs. [6]

Any drug in high enough doses can cause toxic effects. Generally, a large toxic reaction is directly related to the high dose. If the dose is reduced, the toxic effect can be reduced. Every chemical substance is toxic, and the incidence of poisoning is determined by the dose and method of administration. The basic toxicological assessment says that the dose determines whether a chemical contains a lot of poison or not (dosage sola fascit venenum). [7] Many factors determine whether a chemical is toxic, but the right dose is the most important factor. Every chemical substance, including water, can be administered in small doses, which have no effect, or in very large doses, which can cause poisoning and death. In using chemical substances for therapeutic effects, adequate doses are needed to produce maximum pharmacotherapeutic effects. [8]

The synthesis of chemical substances, estimated at 1000 per year, causes toxicology to cover poisons' properties and study the safety of every chemical substance that can enter the body. These chemicals are called xenobiotics (xeno = foreign). Every new chemical must be examined for its toxic properties before it is allowed for widespread use. [9] Based on Meyer's studies, chemical compounds with a mortality yield of more than 50% and less than 1000 ppm are said to have toxic potential. [10]

One of the methods to test for cytotoxic substances is the toxicity test on shrimp larvae from Artemia Salina Leach (Brine Shrimp Lethality Test). This method is often used for pre-screening active compounds in plant extracts because it is cheap, fast, easy (no need for aseptic conditions), and reliable. Brine Shrimp Lethality Test (BSLT) is a method for testing toxic substances and is used as the first bioassay for natural product research. The BSLT method uses Artemia salina Leach larvae as experimental animals. A toxicity test with the BSLT method is an acute toxicity test on the toxic effects of a compound determined in a short time, namely the period of 24 hours after

administration of the test dose. The BSLT procedure can determine the LC50 value of the activity of active plant components against Artemia salina leach larvae. [11; 12] An extract is said to be toxic based on the BSLT method if the LC value is less than 1000 ppm. Research shows a consistent relationship between the toxicity and lethality of brine shrimp in herbal extracts. The BSLT method can be trusted to test the pharmacological activity of natural ingredients. If the natural ingredients are not toxic, the plant can be further investigated to find its other properties using other experimental animals larger than Artemia salina leach larvae, such as mice and rats, in vivo. [13]

Men often use strong drugs to maintain the function of passion or stamina. In some communities, especially in certain areas, strong drugs are very popular with those in those areas. According to the community, drugs using herbs have less toxicity, are affordable, and are easy to obtain. In general, some strong drugs' ingredients contain compounds from palm plant powder, known to stimulate a low libido in men and increase sexual energy. Hawthorne Berry (Frustus crataegi), Hawthorne helps protect against heart disease and to control high blood pressure and high cholesterol. Gingko Biloba Powder is efficacious in helping support the brain, central nervous system, and impotence. Ginkgo improves peripheral circulation and oxygenation. Pulut flour (rice flour) is a great source of gluten-free protein. A good source of protein is essential for achieving peak sexual performance. Panax Ginseng (Root), Ginseng stimulates physical and mental activity and has a positive effect on the sex glands; Panax ginseng herb helps infertility and prevents premature ejaculation. It tends to the formation of blood and sperm, Cayenne Pepper (red chili) is used to prevent strokes and heart attacks by increasing heart work but not increasing blood pressure, besides having the ability to rebuild tissue and also helping reduce fatigue quickly.

Some of the compounds contained in the tested potent drugs are alkaloids, triterpenoids, saponins, and flavonoids. (1) Saponins are a class of combined chemical compounds, one of the secondary metabolites found from natural sources and various plant species. Specifically, saponins are amphipathic glycosides with a soap-suds-like structure that is produced when shaken in an aqueous solution and whose structure consists of one or more hydrophilic glycosides combined with a lipophilic triterpene derivative. [14] (2) Flavonoid compounds or bioflavonoids are the largest phenolic compounds found in nature. Flavonoids are glycoside compounds consisting of sugars bound to flavones. (3) Alkaloids generally contain at least one nitrogen atom, which is basic and is part of a heterocyclic ring. Alkaloids are known to be toxic to other organisms. [15] (4) Triterpenoids are chemical compounds composed of 4 or 5-ring configurations of 30 carbon atoms and some oxygen. Triterpenoids are natural steroid compounds formed by isoprene C5 units via the cytosolic mevalonate pathway to form C30. Cholesterol is an example of a triterpenoid. These compounds can be toxic at certain levels, which in this case, can cause death to experimental animals, namely Artemia salina Leach larvae. [16]

The artemia life cycle can start when the cyst or egg hatches. After 15-20 hours at 25°C, the cyst will hatch into an embryo. Within a few hours, the embryo will still be attached to the skin of the cyst [17]. The embryo will complete its development in the egg phase and turn into free-swimming nauplii. At first, the nauplii will be orange-brown in color because they still contain egg yolks. Newly hatched Artemia cannot eat because its mouth and anus are not yet fully formed. After 12 hours of hatching, they will change their skin and enter the second larval stage. In the second nauplii phase, nauplii will start to eat, with food in the form of microalgae, bacteria, and other organic detritus.

Nauplii are not picky about the type of feed they consume as long as the material is

available in the water at an appropriate size. The larvae will change their skin 15 times before becoming adults within eight days. Adult brine shrimp average about 8 mm in size, although they can reach up to 20 mm under the right conditions. Under these conditions, the adult larvae will reach 500 times compared to the nauplii phase.

RESEARCH METHOD

The research was conducted at the Bogor Agricultural Institute (IPB) Biochemistry Laboratory in November 2015. The materials used in the research were strong herbs obtained from herbal medicine stalls in Bogor. Artemia Salina larvae, reagents used include technical methanol, methanol, chloroform, 2M H2SO4, mayer reagent, wagner reagent, dragendorff reagent, molish reagent, sulfuric acid, chloroform, acetic anhyde, 37% hydrochloric acid, 95% ethanol, HCl concentrated, 5% FeCl3, Mg metal powder, 1% DMSO, NaCl, hexane, ethyl acetate, distilled water. Meanwhile, the tools used for research were analytical balances, stainless tools, test tubes, micropipettes, filter paper, rotary evaporators, and chemical spoons. Toxic effects were obtained from observations by calculating Artemia salina leach larvae's % mortality (mortality) at each concentration. The number of dead Artemia salina leaches in each vial for 24 hours was counted. Percent mortality is obtained by multiplying the ratio by 100%, namely the number of dead larvae divided by the number of initial larvae multiplied by 100% for each replication. Then it was compared with the control, and the results were analyzed to obtain the LC50 price.

$$\%\ \text{death} = \frac{\text{Number of dead larvae}}{\text{Initial total larvae count}} \times 100\%$$

If there are controls that die, the percentage of deaths is determined by the Abbott formula. [15]

$$A1 = \frac{A - B}{10 - B} \times 100\%$$

A1: percentage of deaths after correction
A: percent percentage of dead larvae in the test
B: percentage of larvae mortality in the control

From the percentage of deaths, look for the probit value of each group of test animals through the table, determine the log dose for each group and then graph it with a straight-line equation for the relationship between probit value vs. log concentration, y = bx + a. Where y is the probit value, and x is the log concentration, a line is drawn from the probit value of 5 (= 50% death) to the X axis, and we get the log concentration. The log concentration is antaloged to get the LC50 or LC50 value. It can also be calculated from the straight-line equation by entering the value 5 (probit of 50% mortality of experimental animals) as y so that x is produced as the log concentration value. LC50 is calculated and obtained from the antilog of the x value. [13] The analysis method was carried out using the manual and probit analysis program methods. The manual probit analysis method uses the probit table to estimate the probit value by converting the percent mortality of nauplii at each concentration to the probit value in the table. Then the regression was calculated manually using a calculator. Then as a comparison, the LC50 value was calculated using the probit analysis program to estimate linear regression and manually convert the percent death response to probit. The average LC50 value was obtained through the manual method and the probit analysis program.

RESULT AND DISCUSSION

The number of larvae per test tube with four replications was 40 individuals. The total number of Artemia salina leach larvae used was 120 larvae. The total mortality of the larvae was obtained by adding up the dead larvae at each concentration, while the average mortality of the larvae was obtained by dividing the total mortality of the larvae at each concentration by the number of repetitions carried out, namely four times. Then the percentage of larval mortality was

calculated from the average mortality at each concentration. Observation of the effect of the toxicity of strong herbal medicine X on shrimp larvae was carried out for 24 hours using black paper.

Table 1. Death percentage of Artemia salina leach

Treatment Group	The concentration of strong herbs (ppm)	Number of Artemia salina leach Larvae Mortality in each replication (tail)				Average	% Death
		UI	UII	UIII	UIV		
P1	2000	3	3	2	4	3	30
P2	4000	8	10	8	9	8.75	87.5
k	0	0	0	0	0	0	0

Table 1 shows the number of Artemia salina leach larvae deaths in each test tube at various concentrations. From the table, it can be seen that the highest percentage of mortality in the study was in the treatment group using a concentration of 4000 ppm; this is comparable to the highest average number with a total average of 8.75, which is the highest number of larval deaths in this study and shown in table 1.

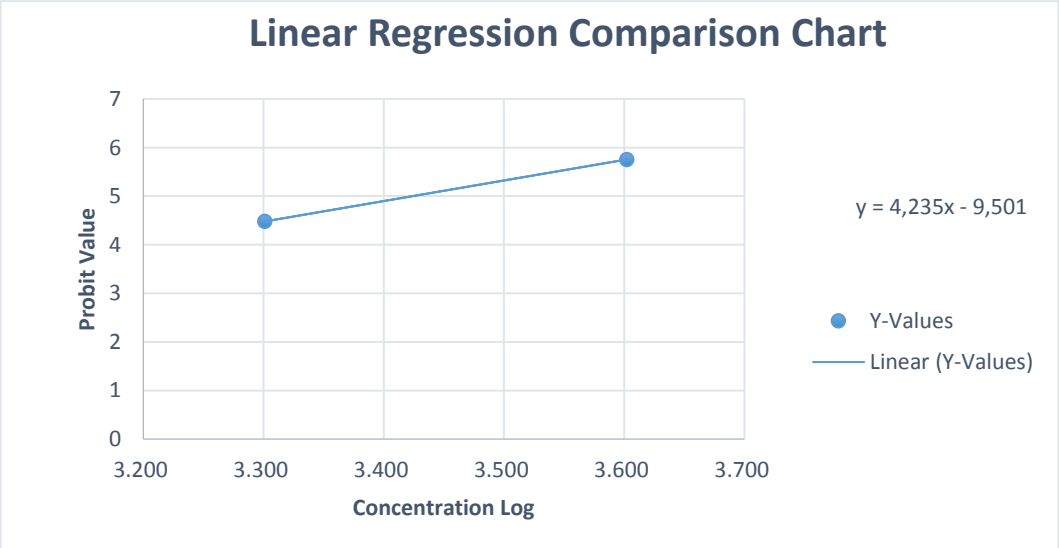
From table 2, which is calculated using Microsoft Excel, the LC50 is 2653.40. In the BSLT method, if the LC50 > 1000 ppm, it can be said that the test substance cannot cause acute toxicity.

Table 2. Log Concentration Determination of LC50

Concentration	Concentration logs (X)	Death percentage	Probit (Y)	X^2	Y^2	XY
2000	3.301	30	4.48	10.897	20.070	14.789
4000	3.602	87.5	5.755	12.975	33.120	20.730
Total	**6.903**		**10.235**	**23.872**	**53.190**	**35.518**

From table 2, which is calculated using Microsoft Excel, the LC50 is 2653.40. In the BSLT method, if the LC50 > 1000 ppm, it can be said that the test substance cannot cause acute toxicity.

Figure 2. Linear Regression Comparison Graph



From the table above, using the Linear Regression Comparison Chart in Microsoft Excel, the log concentrations of LC50 are 3300 and 3600. Compared with manual calculations, the LC50 is not much different from the LC50 probit calculation > 1000 ppm, so the Linear Regression Comparison results do not cause acute toxicity.

B. Phytochemical Analysis

Table 3. Phytochemical Test Results

No	Compound Class	Identification
1	Alkaloids - Meyer - Wagner - Dragendorff	+ + + +
2	-Steroids -Triterpenoids	++ +
4	Saponins (Foam test)	++
5	Flavonoid	+

Information:
+ = small amount of the substance being tested
++ = contains many of the substances tested

This study carried out phytochemical tests, including Alkaloids, Meyer, Wagner, Dragendorff, Steroids, Triterpenoids, Saponins, and Flavonoids. It can be seen from the research results of the phytochemical tests that are presented in table 3. The presence of alkaloids in a substance is because alkaloids can dissolve in polar and non-polar solvents. The type of alkaloid found in the n-hexane fraction is thought to contain more nitrogen atoms which cause it to be more basic and non-polar. In the steroid test a purplish-green crust is formed for the steroid test and a red color for the triterpenoid test. The study found positive steroids and saponins, meaning that a strong drug contains steroids and saponins. In the flavonoid test, a positive result from this reagent was indicated by the formation of foam and a change in the color of the solution to orange this reagent. A positive indicator in the flavonoid test with 10% NaOH reagent is the formation of yellow, red, brown, or green color, indicating the results of the Hal test indicate that the strong herbal medicine contains positive flavonoids. The results of the phytochemical screening showed that the strong jamu contains alkaloids, steroids, saponins (foam test), and flavonoids. Test Steroids and Saponins. Research is stronger because the materials tested in this study probably contain a lot of Saw Palmetto Powder and Panax Ginseng (Root).

CONCLUSION

This study's administration of strong herbal medicine showed no toxicity to Artemia salina leach larvae, as indicated by the LC50 result of 2653.40 ppm according to the BSLT method. The Alkaloid, Meyer, Wagner, Dragendorff, Steroid, Triterpenoid, Saponin, and Flavonoid Phytochemical tests obtained positive results in this study. The steroid and saponin test (foam test) obtained the strongest results. Due to the limitations of the researchers, the researchers used the Brine Shrimp Lethality Test (BSLT) method to see toxic effects because, apart from being cheap, it is easy and has fast results. For other researchers who want to see the toxic effects of certain drugs can use higher species, such as mice, rats, or rabbits.

Declaration by Authors
Ethical Approval: Approved
Acknowledgement: None
Source of Funding: None
Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Dame Joyce Pohan, Randolph Serep Marantuan, Mulyadi Djojosaputro. Toxicity test of strong drug using the BSLT (*Brine Shrimp Lethality Test*) method. *Int J Health Sci Res.* 2023; 13(2):203-209. DOI: *https://doi.org/10.52403/ijhsr.20230228*

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