

Acute and Subchronic Toxicity of Self Nanoemulsifying Drug Delivery Systems (SNEDDS) from Chloroform Bay Leaf Extract (*Eugenia Polyantha* W.) with Palm Kernel Oil as A Carrier

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Abstract. The present study was aimed to study the acute and subchronic toxicity of Self Nanoemulsifying Drug Delivery Systems (SNEDDS) from chloroform bay leaf extract with Palm Kernel Oil as carrier. In acute toxicity test, five groups of rat (n=5/groups) were orally treated with Self Nanoemulsifying Drug Delivery Systems (SNEDDS) from chloroform bay leaf extract with doses at 48, 240, 1200 and 6000 mg/kg/day respectively, then the median lethal dose LD₅₀, adverse effect and mortality were recorded up to 14 days. Meanwhile, in subchronic toxicity study, 4 groups of rats (n=6/group) received by orally treatment of SNEDDS from chloroform bay leaf extract with doses at 91.75; 183.5; 367 mg/kg/day respectively for 28 days, and biochemical, hematological and histopathological change in tissue such as liver, kidney, and pancreatic were determined. The result show that LD₅₀ is 1045.44 mg/kg. Although histopathological examination of most of the organs exhibited no structural changes, some moderate damage was observed in high-dose group animals (367 mg/kg/day). The high dose of SNEDDS extract has shown mild signs of toxicity on organ function test.

1. Introduction

Bay leaf (*Eugenia polyantha*) is one of the plants used to treat diabetes mellitus, beside that it is also known to have potency to cure high blood pressure, high cholesterol, hypertension, gastritis and diarrhea [1]. Ethanolic extract of bay leaf have some active compound such as saponins, triterpenoids, flavonoids, polyphenols, alkaloids, tannins and essential oils consisting of sesquiterpenes, lactones and phenols [2]. Flavonoids lead to decreasing the blood glucose levels [3].

Non polar extract of bay leaf has low solubility and low bioavailability profile. It will influence the therapeutic effect, so that its require a large dose to achieve the optimized therapeutic effect [4]. SNEDDS (*Self-Nanoemulsifying Drug Delivery System*) is one of dosage form to improve bioavailability and solubility profile in the body.

According to previous research reported that SNEDDS of bay leaf chloroform extract using *Palm Kernel Oil* as carrier with compound of surfactant-cosurfactant using Tween 80-PEG (polyethylene glycol) 400 showed characterization of nanoparticle with transmittance values of 81.81% and the average size droplet 218.90 nm [5]. Antidiabetic activity showed that SNEDDS of bay leaf chloroform



extract with Palm Kernell Oil as a carrier to rats at a dose of 91.75 mg/kg BW can lower blood glucose levels equivalent to metformin [6].

The toxicity test consists of 2 types: general toxicity tests include acute toxicity, subchronic and chronic toxicity, and special toxicity tests such as teratogenic test. Acute toxicity test was determined value of LD₅₀ [7]. Herbal medicine requires time to achieve therapeutic effects due to slow drug reactions. In addition, its use repeatedly and in the long term, can allow the emergence of unexpected side effects too, it is necessary to test the subchronic toxicity to know harmful effects that may occur in the long-term use of the drug. Research on the toxicity of bay leaf extract and its dosage form has not been widely studied in Indonesia. One study suggested that the LD₅₀ extract of bay leaf (*Eugenia polyantha W.*) to zebrafish embryos at 0.060 mg/ml [8].

Based on the above description, an acute and subchronic toxicity test of SNEDDS extract of chloroform bay leaf extract is required to determine the level of the dosage in a single dose and repeated dose. The aim of this research was to find out LD₅₀ of SNEDDS bay leaf extract, its toxic symptoms, and to know the effect of SNEDDS preparation of bay leaf extract with repeatable dose for 28 days, profile of weight, SGPT (Serum Glutamic-Pyruvic Transaminase), billirubin and histological profile in liver, pancreas, and kidney of rats.

2. Experimental

2.1 Materials

Materials which were used in this research are bay leaf, male wistar rat, distilled water, chloroform, Palm Kernel Oil (PKO), PEG 400, tween 80, formalin 80%, ethanol 70 % ethanol 95%, ethanol 99.5%, xylol solution, paraffin liquid, Mayer's egg albumin, hematocyclines-xylene, Harri'shematoxylene, and entellan®.

2.2. Procedure

2.2.1. Preparation SNEDDS Bay Leaf Extract with Palm Kernell Oil as a Carrier. Bay leaf simplicia powder was extracted by using maceration method with chloroform solvent for 5 days. Macerate was filtered through glass funnel to separate macerate from simplicia powder. Macerate have been evaporated with rotary evaporator in 55⁰C temperature and 6 rotary speeds until it became concentrated. Each material is weighed and prepared. Chloroform extract of bay leaf (0.15 grams) was added to 5 ml of carrier component, then homogenized with magnetic stirrer for 30 minutes. It was sonicated for 15 minutes, then incubated in water bath at a temperature 45⁰C for 10 minutes. Finally, preparations settling at a temperature of 27⁰C for 24 hours.

Table 1. Formula of SNEDDS

Material	Amount
Bay leaf chloroform extract	0.15 g
Tween 80	1.6 ml
PEG 400	2.4 ml
Palm Kernell Oil	1.0 ml

2.2.2 Animal Preparation. Before testing begin, animals are adapted in the experimental room for seven days. Animals are observed health and behavior. Animals used in the experiment were healthy animals, no weight loss exceeded 10% and showed no behavioral abnormalities and deviations from normal circumstances [9]. Principles of laboratory animal care guidelines were followed and prior permission was sought from the Health Research Ethics Committee for conducting the study (Ref. No. 432/V/HREC/2017).

2.2.3 Acute Toxicity Test. Test animals were used male white rats Wistar strain, age 2-3 months, initial weight 113-171 grams. There were 5 groups (n=5) consisting of 4 test groups with 4 dose variations and 1 control group. Test groups consist of SNEDDS from chloroform bay leaf extract. Four test group were administered 4 doses (48, 240, 1200, and 6000 mg/kg BW) while control group was only given vehicle. Then observed the behavior of animal test after 3-4 hours of treatment, the observation was done again after 24 hours of treatment, observed the presence or absence of animal death test. Observations were made 14 days later to see the reversibility of toxic effects. Noted all behavioral changes that occur in animal testing and determination of LD₅₀ [10].

2.2.4 Subchronic Toxicity Test. Test animals were used male white rats Wistar strain, age 2-3 months, initial weight 90-170 grams. There are 4 groups (n=6) consisting of 3 treatment groups and 1 control group. Determination of doses of subchronic toxicity was based on the lowest dose administered to decrease blood glucose levels in the SNEDDS dosage activity test of 91.75 mg/kg BW [6]. The control group is solution of Na CMC 0.5% comparable with a dose of 91.75 mg/kg BW (KP 91.75); 183.5 mg/kg (KP 183.5); and 367 mg/kg (KP 367). The weight of each animal was measured on days 0, 7, 14, 21 and 28 on subchronic toxicity test, then the percentage of increase weight of each group was calculated. On days 0, 14th, and 28th of animal blood serum test is taken via retro-orbital plexus for SGPT and bilirubin assay using a capillary tube. At the end of the test (day 29), liver, kidney and pancreas of animal has been taken for histological observation [11].

2.2.5 Histopathological Profile. The organ liver, kidney, and pancreas were observed in this study. The organ damage parameter is characterized by permanent cell damage (irreversible). Histologic coloring used HEE type (Hematoxylin eosin). The cell nucleus and cytoplasm so that it can observed the damage of cell components on a specific part. The presence or absence of cell damage depends on the duration and type of necrosis. The stages of necrosis include pycnosis, caryoecsis, and caryolysis.

3. Result and Discussions

3.1 Determination LD₅₀ on Acute Toxicity Test

Test animals that have been previously adapted for one week, were given 5 types of treatment each group with a single dose. After a 14-day observation, 100% dead test animals were only shown at the highest dose of dose of 6000 mg/kg BW. Then 60% of dead animals were shown at a dose level of 1200 mg/kg BW. While in the other group there was no death of the test animal. The number of animal mortality tests was used to calculate LD₅₀ obtained results of 1045.44 mg/kgBW were included in the slightly toxic category based on the range of the toxicity compound. Slightly toxic means that temporary minor injury will result from contact with, or absorption of a small to moderate amount by a healthy adult. Larger quantities of exposure could, however, cause greater damage. The results of this acute toxicity test show the overall toxicity of the SNEDDS preparation, both the active substance and the carrier as a whole. The high level of toxicity in this study can not be separated from the role of the concentration of each component of the preparation in harmony with the higher dose given. In addition, the volume of dosage also plays a role in causing death at high doses. If there are overvolume, it will cause overhydration of the test animal cells consequently the cell will expand and over time will rupture causing cell death.

Table 2. Percentage of Mortality in Acute Toxicity Test

Groups	Number of rats	Number of Mortality	Percentage of Mortality
Control	5	0	0
48 mg/kg	5	0	0
240 mg/kg	5	0	0
1200 mg/kg	5	3	60%
6000 mg/kg	5	5	100 %

3.2 Subchronic Toxicity Test

3.2.1 Weight profile

Table 3. Percentage Increase of Weight

Day	Control (%)	KP 91.75 (%)	KP 183.5 (%)	KP 367 (%)
0	-	-	-	-
7	2.09	10.24	7.79	3.78
14	2.84	12.77	12.70	6.06
21	17.37	6.75	13.70	21.20
28	8.01	4.39	5.73	6.82

There are many factors can cause weight gain such as limited activity and food which contain carbohydrates and fats. Intake of feed is not converted into energy but will be converted into body fat [12].

3.2.2 Profile of SGPT (Serum Glutamic-Pyruvic Transaminase)

Table 4. Value of SGPT Each Groups

Groups	Day		
	0	14	28
Control (Unit/L)	58.917±6.503	67.950±16.216	58.517±15.726
KP 91.75 (Unit/L)	55.600±9.967	66.483±9.408	59.483±13.703
KP 183.5 (Unit/L)	53.050±5.731	60.417±12.647	72.033±8.536
KP 367 (Unit/L)	51.750±13.779	80.720±12.920	68.920±18.830

Normally, value of SGPT for rate male between 42.9-67.4 U/l. The result showed that the value of SGPT has increased in all groups. Measurements of SGPT were conducted in order to support the observation histopatologic of liver. SGPT is a specific enzyme in the liver. Its present only in the liver so it can be a specific parameter to determine the condition of liver function. Generally, increasing value of SGPT until exceed upper limit, showed likelihood of liver damage.

3.2.3 Profile of Billirubin

Table 5. Value of Billirubin Each Groups

Groups	Day		
	0	14	28
Control (mg/dl)	0.540±0.127	0.492±0.331	0.345±0.055
KP 91.75 (mg/dl)	0.522±0.094	0.590±0.199	0.455±0.109
KP 183.5 (mg/dl)	0.538±0.175	0.527±0.169	0.368±0.077
KP 367 (mg/dl)	0.458±0.179	0.454±0.059	0.512±0.115

Normally, value of billirubin between 0.2-0.55mg/dl. The value of bilirubin in each group can be affected by the magnitude of red blood cell reshuffle. Differences in red blood cells are reorganized in each individual to produce different values of bilirubin, this is not independent of the adaptation of animals tested against foreign substances. Overall, the presence of foreign substances that were pawned on test animals did not affect bilirubin levels.

3.2.4 Histopathological Profile

Table 6. Percentage of Total Damage Cells

Groups	Total Damage in 100 cells			Percentage of Damage (%)
	Hepar	Pancreatic	Kidney	
Control	20	19	7	15.33 %
KP 91.75	12	12	11	11.67 %
KP 183.5	23	27	15	21.67 %
KP 367	45	39	21	35 %

The toxicity of nano-sized material came from the size, surface wide, composition, and nanomaterial size [13]. The most important mechanism from nanomaterial toxicity in vitro and in vivo is related to the oxidative stress induction by free-radical formulation [14]. Increasing of Reactive Oxygen Species (ROS) can cause oxidative stress condition that causes the occurrences of tissue damage in the whole biological membrane by protein attack, lipid or fat, nucleate acid, and *glico-conjugat* [15]. Non-ionic surfactants are often used as carriers of nanomedicine preparations because it is less toxic, not cause hemolytic, not irritation and can maintain the pH of a liquid [16]. The study mentions surfactant surface tension above 35 mN/m has a low toxicity, whereas the surface tension of tween 80 at the study 43.2 mN/m [17]. Tween 80 has value of LD₅₀ 25 g/kg orally and 4.5 g/kg intravenous in rat [18]. Previous study has been conducted that LD₅₀ of PEG 400 orally >10g/kg and did not cause significant side effects with repeated administration for two years in mice at a dose of 20 mg/kg/day, but with higher doses showing the low effect on growth and swelling of the liver [19]. Basically, each compound is non-toxic but in use in the long term will still cause side effects. The observation of histopathology blood smear on pancreas, hepar, and kidney after giving for 28 days shows the minor damage in form of cell degeneration. Based on various sources mentioned each organ largely suffered only minor damage (control group, KP 91.75 and KP 183.5) and moderate damage (KP 367).

Cell degeneration can be cellular swelling, picnosis, caryorecsis, and caryolisis. The occurrence of cell degeneration can cause the changes of structure and cell shape that cause inability of cell to function normally. Damage of cells in each organ is still at cellular swelling and picnosis phase. Swelling of the endoplasmic reticulum from increased cell water, one of the earliest ultrastructural changes observed in injured cells, is reversible. Picnosis or core corrugating is a cytoplasm homogenization and eosin cordage increase by the damage intercytoplasmic protein. In caryoecsis, the core cells that experience picnosis is fragmentized. In caryolisis, chromatine become pale. This change is the reflexion of DNA activity because of the cell acid degree reduction [20]. In contrast, picnosis, caryorecsis, and caryolisis are all nuclear signs of cell death and represent irreversible changes.

4. Conclusions

Overall the study of acute and subchronic toxicity test preparation SNEDDS bay leaf chloroform extract showed LD₅₀ have amounted in 1045.44 mg/kg BW. Subchronic toxicity test results showed a minor damage to the liver, kidneys and pancreas in 183.5 mg/kg rat weight and moderate damage in a dose of 367 mg/kg as indicated by the increased value of SGPT and billirubin. In conclusion, SNEDDS bay leaf chloroform extract used in this study is possible developed as a future drug, because the damage to the body caused by SNEDDS extract is minor and not permanent, considering the dose.

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