

# Acute And Subchronic Toxicity Studies Of SNEDDS (Self Nanoemulsifying Drug Delivery Systems) From Ethyl Acetate Extract Of Bay Leaf (*Eugenia polyantha* W.) with Virgin Coconut Oil As Oil Phase

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**Abstract.** Bay leaf (*Eugenia polyantha*) is widely used as an alternative therapy for diabetic and hypercholesterol. However, the administration of the extract has a low oral bioavailability, therefore it is prepared by Self Nanoemulsifying Drug Delivery Systems (SNEDDS) ethyl acetate extract of bay leaf. Therefore, acute and subchronic toxicity test is required. The toxicity test performed was an experimental study, including acute and subchronic toxicity tests. Animal experiments were used using Wistar strain rats. Acute toxicity test using 5 groups (n=5) consisted of 1 control group and 4 groups of SNEDDS dose with 48 mg/kgBW 240 mg/kg, 1200 mg/kg, and 6000 mg/kg, while for subchronic toxicity test with 1 group control and 3 groups of doses of SNEDDS with dose group variation 91.75 mg/kgBW, 183.5 mg/kg, and 367 mg/kg. Duration of observation at acute toxicity test for 14 days while for subchronic toxicity test for 28 days with continuous SNEDDS dosage. The results of the acute toxicity test showed toxic symptoms and obtained median lethal dose (LD<sub>50</sub>) values from SNEDDS from ethyl acetate extract of bay leaf 1409.30 mg/kgBW belonging to slightly toxic category. Subchronic toxicity studies show that the test drug has minor damage in liver and kidneys and moderate damage in pancreas.

## 1. Introduction

Bay leaf has been used for the treatment of high cholesterol, diabetes (diabetes mellitus), high blood pressure (hypertension), gastritis, diarrhea and gout [1]. Bay leaf extract has a large molecular size and low stability in the body. Tween 80 has been used because it has high value of Hydrophilic Lipophilic Balance (HLB), a good wetting agent and a good emulsifying agent [2]. PEG 400 was selected as cosurfactant which is help solubilize hydrophilic surfactants as well as drugs in an oil base [3]. Cosurfactant determines the emulsification time in the medium as well as the size of the nanoemulsion due to cosurfactant molecule to place its position between surfactants. Cosurfactant is an amphiphilic compound such as propylene glycol, polyethylene glycol, and glycol esters having affinity to the water and oil phases [4]. The carrier which often used in the manufacture of SNEDDS is vegetable oil. Vegetable oil was chosen because it was more easily degraded by microorganisms so it is more

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environmentally friendly [5]. Virgin Coconut Oil (VCO) has long-chain fatty acids that contain a lot of linoleic acid. Long-chain triglycerides have the advantage of being able to increase the transport of drugs through the lymphatics thereby reducing the first pass metabolism. SNEDDS ethyl acetate extract of bay leaf with VCO carrier has a transmittance value of 90.95% and these results indicated the high clarity of nanoemulsion. That has a droplet size range from 50-100 nm. SNEDDS ethyl acetate extract of bay leaf with VCO carrier has activity for antidiabetic type II insulin resistant. It can decrease blood glucose level equivalent to metformin [6] [7].

Toxicity studies are required to assess potential hazards to humans through the acute, subchronic, and chronic exposure of laboratory animals to pesticides. Acute toxicity studies provide information on the potential for health hazards that may arise as result of short-term exposure. Determination of acute oral, dermal, and inhalation toxicity is usually the initial step in evaluating the toxic characteristics of a drug. In each of test, the animal is exposed to the test material once on one day. One study of acute toxicity test showed that the LD<sub>50</sub> bay leaf extract to zebrafish embryos at 0.060 mg/ml [8].

Subchronic exposures do not elicit effects that have a long latency period. However, they do provide information on health hazards that may result from repeated exposures to a drug. Subchronic tests also provide information necessary to select proper dose levels for chronic studies, especially for toxicity studies for which an maximum tolerated dose (MTD) must be selected.

Based on the description, an acute and subchronic toxicity test of SNEDDS ethyl acetate extract of bay leaf is required to determine the level of the dosage in a single dose and repeated dose which caused toxicity. The aim of this research was to determined LD<sub>50</sub>, toxic symptoms, the effect of repeated dose for 28 days, profile of weight animal test, SGPT (Serum Glutamic-Pyruvic Transaminase) value, billirubin value, and histological profile of liver, pancreas, and kidney.

## 2. Experimental

### 2.1 Materials

Materials which were used in this research are are bay leaf obtained from Sukoharjo, male wistar rat (125-170 grams), distilled water (Brataco), chloroform (Brataco), ethyl acetate (Brataco), Virgin Coconut Oil (Agung Jaya), PEG 400 (Agung Jaya), tween 80 (Agung Jaya), formalin 80% (Brataco), ethanol 70 % (Brataco), ethanol 95% (Brataco), ethanol 99,5% (E Merck), xylol solution (E Merck), paraffin liquid (Brataco), Mayer's egg albumin (E merck), hematocyclines-xylene (E Merck), Harri's hematoxylene (E Merck), and entellan® (E Merck).

### 2.2. Procedure

**2.2.1. Preparation of SNEDDS.** 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. The powder then remaceration with ethyl acetate. It was evaporated in 55°C temperature and rotary speed at 60 rpm until it became concentrated. Each material is weighed and prepared. Ethyl acetate extract of bay leaf (0.15 grams) was added into 5 mL of carrier component, then it was homogenized with magnetic stirrer for 30 minutes. It was sonicated for 15 minutes, incubated in waterbath 45 °C for 10 minutes. Finally, it was kept at 27 °C for 24 hours.

**Table 1.** Formula of SNEDDS [6]

Material	Amount
Bay leaf ethyl acetate extract	0.15 g
Tween 80	1.33 mL
PEG 400	2.67 mL
Virgin Coconut Oil	1.0 mL

**2.2.2 Animal Preparation.** Before proceeding for toxicity studies animals, Wistar strain rats (125-170g) reared at animal house of Sub Biology Laboratory Sebelas Maret University, were selected and housed separately and kept under strict observation for a period of one week with free access to food and water. Any animal showing sluggish movement or any sign of illness was rejected. 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. 433/V/HREC/2017).

**2.2.3. Acute Toxicity Test.** Test animals were used male white rats Wistar strain, age 2-3 months, initial weight 125-170 grams. There were 5 groups (n=5) consisting of 4 treatment groups (SNEDDS ethyl acetate extract of bay leaf) with 4 dose variations and 1 control group. For treatment groups, group I with dose of 48 mg/kg BW; Group II 240 mg/kg; Group III 1200 mg/kg and group IV 6000 mg/kg while the control group is SNEDDS without extract. The reversibility of toxic effects was observed for 14 days. All behavioral changes of animal was noted [9].

**2.2.4. Subchronic Toxicity Test.** Test animals were used Wistar male white rats, age 2-3 months, initial weight 125-170 grams. There are 4 groups 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 / kgBW [7]. Three test group were administered 3 dose (91.75, 183.5, and 367 mg/kg BW) while control group was only given vehicle. The weight of each animal was measured every week on subchronic toxicity test, then the percentage of increase weight of each group was calculated. Animal blood serum test was taken via retro-orbital plexus for SGPT and bilirubin assay using a capillary tube on days 0, 14<sup>th</sup>, and 28<sup>th</sup>. Histological observation has been done at day 29<sup>th</sup> [10].

### 3. Result and Discussions

#### 3.1 Determination LD<sub>50</sub> 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 / kgBW. The median lethal dose (LD<sub>50</sub>) was 1409.30 mg/kg BW for male rats. The result showed that SNEDDS is minor toxic for oral administration. Minor toxic means that temporary minor injury will result from contact with, or absorption of a small to moderate amount by a healthy adult. Rats and humans have similarity, which is both of them are mammals. If SNEDDS extract is minor toxic in rats, hopefully so is in human. The others plant which have antidiabetic effect are minor toxic such as crude extract of *Vernonia amygdalina* (LD<sub>50</sub> 1265 mg/kg in rat, oral) [11], and *Physalis peruviana* L. (LD<sub>50</sub> 1280 mg/kg in guinea-pig, oral) [12].

The maximum tolerated dose is higher than the dose required having antidiabetic effects. However, like crude extract which has been recognized toxic, the use of SNEDDS should also be carefully adjusted or discouraged.

**Table 2.** Percentage of Mortality in Acute Toxicity Test

Groups	Percentage of Mortality
Control	0
I	0
II	0
III	40%
IV	100 %

### 3.2 Subchronic Toxicity Test

**Table 3.** Body Weight Profile

Day	Body weight of Rats (gram)			
	Control	91.75 mg/kg	183.5 mg/kg	367 mg/kg
0	140.83±12.70	120.33±21.45	131.17±13.99	123.67±10.31
4	143.83±13.17	128.33±21.62	135.50±13.65	126.50±8.50
7	146.50±13.31	135.33±23.02	139.33±14.39	128.33±7.66
11	150.00±14.30	141.83±24.31	145.00±13.70	128.60±7.44
14	152.23±15.56	148.33±26.68	149.50±13.81	132.80±6.02
18	162.83±16.24	158.17±33.14	154.33±13.00	140.60±10.97
21	170.50±17.03	161.17±34.31	155.83±13.73	143.20±14.57
28	179.83±19.72	168.33±37.25	159.17±14.82	155.25±15.41
Increase of weight (%)	27.69	39.89	21.34	25.53

Based on the table 3, dose 91.75 mg/kg give highest of increase weight than other groups. The alteration of body weight gain of the treatment groups compared to the control group would reflect the toxicity of the substance [13]. Significant difference in body weights between the treatment and control group may occur in the absence of any morphological changes [14].

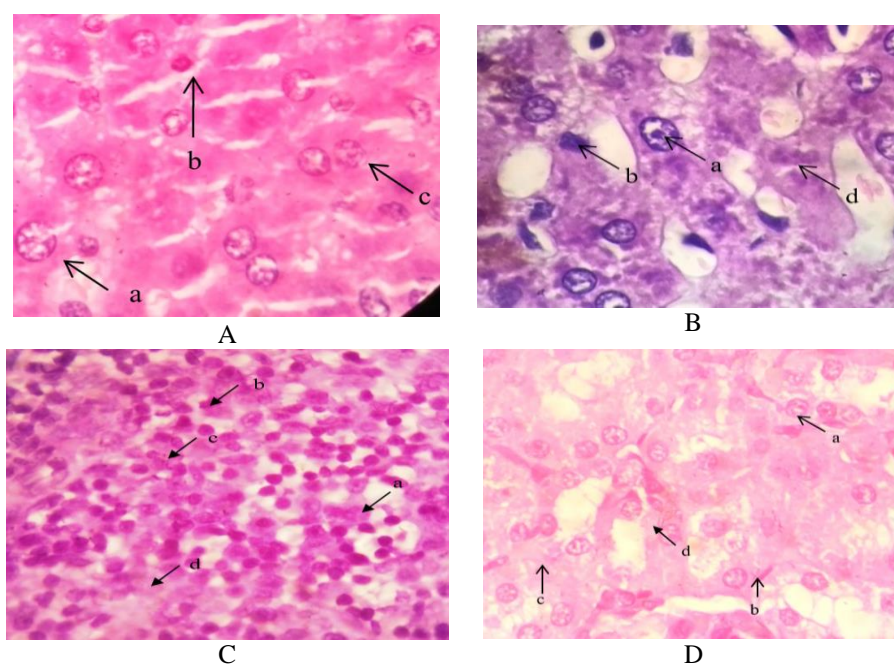
**Table 4.** Value of SGPT each groups

Groups	Day			Criteria
	0 (Unit/L)	14 (Unit/L)	28 (Unit/L)	
Control	57.25±11.00	63.63±4.60	75.88±8.90	<b>42.9-67.4 U/L</b>
91.75 mg/kg	53.32±3.07	51.33±7.14	69.08±16.45	
183.5 mg/kg	58.57±15.60	70.00±16.01	71.60±8.98	
367 mg/kg	51.23±4.76	72.55±14.53	79.10±10.20	

**Table 5.** Value of Billirubin each groups

Groups	Day			Criteria
	0 (mg/dl)	14 (mg/dl)	28 (mg/dl)	
Control	0.45±0.06	0.47±0.05	0.44±0.15	<b>0.2-0.55 mg/dl</b>
91.75 mg/kg	0.49±0.12	0.57±0.14	0.32±0.06	
183.5 mg/kg	0.50±0.11	0.55±0.05	0.38±0.06	
367 mg/kg	0.49±0.05	0.58±0.09	0.36±0.09	

Elevation in SGPT and billirubin is an indicator of liver and heart damage[15]. Based on the table 4 and 5, after 28 days of extract administration there were no significantly different in the values of SGPT and billirubin in male rats test groups compared to control groups. It indicates the extract was not toxic for liver and did not affected lipid metabolism.



**Figure 1.** Photomicrograph of tissues in 28 days of SNEDDS ethyl acetate extract of bay leaf treatment. (A) control groups, (B) SNEDDS 367 mg/kg BW in hepar tissues, (C) SNEDDS 367 mg/kg in pancreatic tissues, (D) SNEDDS 367 mg/kg in kidney tissues  
400x

**Table 6.** Percentage of Total Damage Cells

Groups	Total Damage in 100 cells			Percentage of Damage
	Kidney	Pancreas	Liver	
Control	13	25	18	18.67 %
91.75 mg/kg	15	33	9	19.00 %
183.5 mg/kg	22	46	21	29.67 %
367 mg/kg	30	44	28	34.00 %

The four weeks daily treatment with the SNEDDS at three doses did not show any toxicity related mortalities and changes in general health, behaviour, motor activities, and growth. Hence, it can be concluded that SNEDDS has no effect on growth and normal functions of the rat at low doses [16].

The present histopathological examination indicated that some of the pancreas sections obtained from rat treated SNEDDS 367 mg/kg body weight test dose showed picnosis and distorted general architecture. Picnosis, which appeared chiefly in pancreas sections of rat treated at SNEDDS 367 mg/kg dose, occurs when a cell receives a signal to initiate apoptosis [17-20]. Damage from toxic or immunologic insult may cause hydropic degeneration of hepatocytes in which cells take on a swollen, edematous appearance with irregularly clumped cytoplasm and vacuolations [20]. The presence of picnosis and protoplasmic changes like vacuolation in swollen hepatocytes possibly points potential morphologic evidence of degeneration [17-20].

In subchronic toxicity study, the general histological architecture of kidney and liver sections of rat in all experiment groups was not affected. However, some sections of kidney obtained from rat treated with the SNEDDS 367 mg/kg body weight test dose were showed cellular swelling and picnosis. Being focal and minor, the observed changes are not necessary indicators of renal disease or injury [21]. However, further toxicological investigation is recommended on other vital internal organs as well as on non-rodent species to confirm this finding.



SNEDDS makes macrophages entering it in cells and degradation processes in the cell may have a cytotoxic effect [22]. Small-sized nanomaterials will accumulate in certain tissues (the spleen, liver, lungs, and kidneys) which will cause impaired biological function of each of these tissues [23]. The mechanisms that occur in nanomaterial toxicity are related to oxidative stress induction by free radical formation [24]. Free radicals are released by phagocyte cells in response to foreign bodies, lack of antioxidants, and environmental factors. The organ that is most frequently affected by the nanomaterial is the liver and spleen due to phagocyte cell prevalence in the endothelial reticulum. In addition, the organs with high blood flow such as kidneys and lungs [25]. Intracellular nanoemulsions may interact with different components, especially with mitochondria and nucleus, which are the main sources of cell toxicity, induced apoptosis, and ROS formation [26]. SNEDDS at relatively lower doses does not produce obvious toxic effects after acute and prolonged oral administration in rat. However, further investigation is needed to confirm this.

#### 4. Conclusions

The high dose of SNEDDS ethyl acetate extract of bay leaf may cause intoxication. The use of the SNEDDS should also be carefully adjusted or discouraged.

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#### References

- [1] Utami P and Tyas D E 2013 *The Miracle of Herbs* (Jakarta: Agro Media)
- [2] Salager J L 2002 *Surfactants Types and Uses Laboratory Of Formulation, Interfaces, Rheology and Process* (Colombia:Universidad De Los Andes)
- [3] Amrutkar C, Salunkhe K and Chaudhari S 2014 *World Journal of Pharmaceutical Research* **3** (4): 2137-2151
- [4] Makadia H A, Bhatt A Y, Parmar R B, Paun J S and Tank H M 2013 *Asian J. Pharm. Res.* **3** 21–27
- [5] Patel J Kevin G Patel A Raval M and Sheth N 2011 *Int. J. Pharm. Investigation* **1** 112–118
- [6] Prihapsara F, Harini M, Widiyani T, Artanti A N and Ani I L 2017 *IOP Conference Series: Materials Science and Engineering* **176** (1)
- [7] Harini M, Widiyani T and Prihapsara F 2016 Development Nanoherbal of Bay Leaf as an Antidiabetic Agent *Research Report* Sebelas Maret University
- [8] Ismail H F, Zanariah H, Wong T N, Nur S R, Zainudin A N and Majid F A A 2017 *Journal of Traditional and Complementary Medicine* **1** 14
- [9] Sari W P 2010 Acute Toxicity Test Mixture Ethanol Extract Betel leaf ( *Piper betle* L.) And Dry Extract Gambir ( *Uncaria Gambir* R.) Mice Against White Males *Thesis* University of Islamic State Jakarta
- [10] Krysanti A and Widjanarko S B 2014 *Journal of Food and Agro Industry* **2** 1
- [11] Michael U A, David B U, Theophine C O, Philip F U, Ogochukwu A M and Benson V A 2010 *Journal of Basic and Clinical Pharmacy* **1** (3)
- [12] Kasali F M, Kadima J N, Mpiana P T, Ngbolua K and Tshibangu D T 2013 *Asian. Pac. J. Trop. Biomed.* **3** (11)
- [13] Michael J D and Manfred A H 2014 *Handbook of Toxicology 3<sup>rd</sup> Edition* (United States : CRC Press Inc.)
- [14] Bailey S A, Zidell R H and Perry R W 2004 *Toxicol. Pathol.* **32**(4) : 448-466
- [15] Crook M A 2006 *Clinical Chemistry and Metabolic Medicine* (London : Hodder Arnold) p 426
- [16] OECD 2001 *Guidelines for Testing of Chemicals, Acute Oral Toxicities up and down Procedure* 425: 1-26
- [17] Vanden B T, Vanlangenakker N, Parthoens E, Deckers W and Devos M 2010 *Cell Death and Differentiation* **17**: 922-930.

- [18] Trump B F, Berezesky I K, Chang S H and Phelps P C 1997 *Toxicol. Pathol.* **1** (8)
- [19] Kerr J F, Wyllie A H and Currie AR 1972 *Br. J. Cancer* **4** (57)
- [20] Kumar V, Cotran R and Robbins S 2002 *Robbins Basic Pathology 7<sup>th</sup> Edition* (Philadelphia: Elsevier Saunders)
- [21] Rose B D 1987 *Pathophysiology of Renal Disease 2<sup>nd</sup> Edition* (New York : McGraw-Hill)
- [22] Rawat, M, Singh D and Saraf S 2006 *Biological and Pharmaceutical Bulletin* **29(9)** : 1790-1798.
- [23] Nel A, Xia T, Madler L and Li N 2006 *Science* 311: 622-627
- [24] Chang, H W, Hsu P C and Tsai Y C 2012 Ag/Carbon Nanotubes for Surface-Enhanced Raman Scattering (Berlin: Springer) p119-135
- [25] Clichici S and Filip A 2015 *License In Tech.* 93-121
- [26] Aillon K L, Xie Y, El G N, Berkland C J and Forrest M L 2009 *Adv. Drug Deliv. Rev.* **61(6)** : 457-466