Acute toxiticy test of high doses of Detam 1 soybean (Glycine max L.merr) extract, Jati belanda (Guazuma ulmifolia) leaves and their combination

DOI: 10.22435/hsji.v8i2.7381.124-132

Meilinah Hidayat[1], Sijani Prahastuti[2], Ellya R Delima[2], Liasisca Setiawati[3], Andreanus A Soemardji[4]

[1]Nutrition Department Maranatha Christian University Indonesia
[2]Biochemistry Department Maranatha Christian University Indonesia
[3]Maranatha Christian University Indonesia
[4]Toxicology Department, School of Pharmacy, Institut Teknologi Bandung Indonesia

Corresponding address: Meilinah Hidayat
Email: mellahidayat@yahoo.com

Received: August 10, 2017; Revised: October 31, 2017; Accepted: November 21, 2017

Abstrak

Latar Belakang: *Sebagai terapi antiobesitas kombinasi ekstrak kedelai Detam-1 dan daun Jati Belanda haruslah aman dan bebas dari bahan toksik. Untuk membuktikan keamanan ekstrak tumbuhan obat, diperlukan uji toksisitas akut. Tes ini terdiri dari: nilai LD50, perilaku, berat organ (BO), dan indeks organ (IO) mencit Swiss Webster setelah diberi ekstrak etanol kedelai Detam-1 (EEKD), ekstrak etanol Jati Belanda (EEJB) dan kombinasinya.*

Metode: *Penelitian eksperimental sejati dengan rancangan acak lengkap sesuai ketentuan BPOM tahun 2014 adalah metode penelitian ini. Dua puluh mencit betina Swiss Webster dibagi menjadi empat kelompok perlakuan. Kelompok 1 (kontrol negatif), 2 (EEKD 2.000 mg / kgBB), 3 (EEJB 2.000 mg / kgBB), dan 4 (kombinasi EEKD dan EEJB 1: 2 sebesar 2000 mg / kgBB). Data BO dan IO dianalisis dengan uji t tidak berpasangan dengan* α *= 0,05.*

Hasil: *Tidak ada gejala mortalitas dan toksisitas pada semua kelompok, semua mencit berperilaku normal, tidak ada perbedaan bermakna dari BO dan IO dari delapan organ utama semua grup secara statistik (p> 0,05) kecuali paru-paru, hati dan limpa pada kelompok 2.*

Kesimpulan: *Nilai LD50 dari EEKD, EEJB dan kombinasinya seluruhnya di atas 2.000 mg/kgBB, tidak menimbulkan perubahan perilaku mencit; BO dan IO pada mencit yang diberi dosis sangat tinggi EEKD, EEJB dan kombinasinya.* ***(Health Science Journal of Indonesia 2017;8(2):124-32)***

Kata kunci*: LD_{50}, perilaku, bobot organ, indeks organ, kedelai Detam-1, daun Jati Belanda*

Abstract

Background: As an antiobesity therapy the combination of Detam-1 soybean extract and Jati Belanda leaves extract should be safe and free from toxic material. In order to prove the safety of both medicinal plant's extract, acute toxicity test is needed. The test consists of : LD50 value, behaviour, organ weight (OW), and organ index (OI) of Webster Swiss mice after feeding with ethanol extract of Detam-1 soybean (EEDS), ethanol extract of Jati Belanda (EEJB) and its combination. The aim of this research was to know the value of Lethal Dose (LD) 50, behavior, organ weight (OW), and organ index (OI) in Swiss Webster mice after administered of ethanol extract of Detam-1 soybean (EEDS), ethanol extract of Jati Belanda (EEJB) and their combination.

Methods: True experimental study with complete randomized design in accordance with BPOM 2014 was the methods of this study. Twenty Swiss Webster female mice were divided into four treatment groups. Group 1 (negative control), 2 EEDS (2,000 mg / kgBB), 3 (EEJB 2,000 mg / kgBW), and 4 (EEDS and EEJB 1: 2 combination of 2000 mg / kgBW). OW and OI data were analyzed by independent t test with $\alpha = 0.05$.

Results: There were no symptom of mortality and toxicity in all groups, all of mice behave normally, statistically no significant differences in OW and OI of the eight major organs of all groups ($p > 0.05$) except lung, liver and spleen in group 2.

Conclusion: The LD50 value of EEDS, EEJB and their combinations entirely above 2,000 mg/kgBW, no changing on the behavior, OW and OI in mice which given very high dose of EEDS, EEJB and their combination. ***(Health Science Journal of Indonesia 2017;8(2):124-32)***

Keywords : LD_{50}, behavior, organ weight, organ index, Detam-1 soybean, Jati Belanda

Drugs are chemical substances that can affect the life processes and metabolism of an organism. During this time people consider that traditional medicine is relatively safe and will not cause side effects because it comes from nature. But in fact not some traditional drugs expose side effects, even unwanted toxic effects.[1,2]

Jati Belanda (*Guazuma ulmifolia*) leaves and black soybeans (*Glycine max* L.merr) are plants that have various benefits. Empirically Jati Belanda seeds are used by the community as anti-diarrhea; its leaves as a slimming tea, and antidiabetes; and the bark is used for pneumonia, hemorrhoids, cough and bronchitis[3] whereas black soybean seeds are used as food, animal feed, and industrial raw materials.[4]

Previous studies have shown that ethanol extract of soybean seeds Detam 1 (EEDS) and Jati Belanda leaves ethanol extract (EEJB) can inhibit pancreatic lipase enzymes so they have an effect of lowering total cholesterol levels, and can cause weight loss.[5] However, in addition to its usefulness, previous research showed the presence of hepatotoxic side effects on a single EEJB administration, whereas the single effect of EEDS actually leads to an improvement in histopathological features of the liver. Histopathological features of the liver in administration of the combination extract were much better than the single EEJB administration because in EEDS contains isoflavone, which is a powerful antioxidant and could decrease oxidative stress.[6,7]

As an antiobesity Detam-1 soybean in combination with Jati Belanda leaf should be safe and free from toxic substances. Previous study, subchronic toxicity test as antiobesity showed that low dose combinations (150 mg/kgBW), moderate (300 mg/kgBW) and high doses (600 mg/kgBW) were safe. But the maximum deadly dose for living things is still unknown, therefore an acute toxicity effect test with very high dose (2,000 mg/kgBW) is required. In general, an acute toxicity test is performed before performing a chronic toxicity test. Main parameters of acute toxicity test i.e. LD50 and other supporting parameters are behavioral changes, organ weight (OW), and organ index (OI). Lethal dose 50 (LD50) is the dose that causes death in 50% of experimental animals. The toxic effects of a compound can be determined by LD50 test performed in a short span of time by giving the compound within a certain dose. Acute toxicity tests may show symptoms of toxicity that will appear in behavioral changes, OW, and OI indexes that under certain circumstances can lead to death.[8] This study was conducted in accordance with the rules issued by the Republic of Indonesia's Food and Drug Supervisory Agency (BPOM) number 7 of 2014 on non-clinical toxicity test guidelines *in vivo*.[9]

METHODS

The tools used in this research were evaporator, rotary vacuum evaporator, platform, macerator, Pasteur pipette, oral sonde, digital scales, analytical scales, stopwatch, mice cage, CO2 chamber,. The sample used were soybean seed Detam 1 (from Balitkabi Malang), Jati Belanda leaves (from Bumi Herbal Dago, North Bandung), 95% ethanol, aquadest, 0,5% Sodium Carboxymethyl cellulose (Na CMC 0.5%), husk grass, and mice food. The ethanol extracts was obtained from simple maceration procedures.

Subjects were 20 healthy Swiss Webster female mice, nullipara, not pregnant, aged 8-12 weeks weighing 25-40 grams. The reason female mice were chosen is that female mice are more sensitive and sensitive to various stimuli and changes.[10] Mice were adapted for 5 days before treatment. Prior to treatment, the mice were fasted for 3-4 hours- but allowed to take drinking water. Mice were weighed and given oral test preparations using gastric sonde. After 1-2 hours of treatment- mice were fed again.

Mice were divided into 4 groups: (1) Group I (negative control) NaCl 0.5% 1 mL / 20 gram. (2) Group II (single EEDS dose 2,000 mg / kgBB), (3) Group III (single EEJB dose 2.000 mg / kgBB), and (4) Group IV combination EEDS and EEJB (1: 2) 2000 mg / kgBB mice. In our previous study, subchronic toxicity test used 3 doses, low 150 mg/kgBW, medim 300 mg/kgBW and high 600 mg/kgBW; all samples of combination of EEDS and EEJB in 1:2 proportional.

Provision of test preparation in this experiment was attempted at the highest dose level, ie 2,000 mg/kgBW of mice on the premise that soy is a common food ingredient and the substance content in EEDS is not potentially toxic, and it is supported by previous research results.[11] Yet we have to know the maximum deadly dose for living things, therefore this acute toxicity effect test with very high dose (2,000 mg/kgBW) is performed. Subchronical toxicity of combination of Detam 1 soybean and Jati Belanda ethanol extract had no toxic effect on renal function and did not cause macro and micropathological damage to renal organ of Wistar rats after treatment of low (150 mg/kgBW), medium (300 mg/kgBW) and high dose (600 mg/kgBW) for 90 days, characterized

by no differences in mean weight of renal organ between treatment group and control group and low dosage (converted dose to human: 8.400 mg) was also safe and did not change renal histopathologic features.[11] Test preparations methods is based on the rules which set by the Organization for Economic Co-operation and Development (OECD) Guideline For Testing of Chemicals Acute Oral Toxicity - Fixed Dose Procedure (guideline number 420) 2001.[12]

Individual mice were observed at 30 minutes after administration of the test preparation, subsequently after 1, 2, 4, and 24 hours of administration and afterward once a day for 14 days. Observations were daily performed for 14 days for the cardiovascular system, respiration, somatomotoric, skin and feathers, mucosae, and eyes. Particular attention was provided when there were disorders or diseases, such as tremor, seizures, salivation, diarrhea, lethargy, weakness, deep sleep or coma.[12] Observations included the time of incidence, the loss of toxic symptoms and the occurrence of death. Any dying mice, would be killed and counted as a dead animal. Mice were weighed 2 times in 1 week.

At the end of the study, all mice both which dead during the test period and lived at the end of the study, were all killed and autopsied to evaluate the presence of toxicity symptoms, to perform macropathology of each organ, and determine the absolute organ weight (OW) and relative organ weight or Index organ (IO). The value of relative organ weight were obtained from the formula: absolute organ weight divided by body weight of the experimental animal

This study has obtained ethical approval from the Research Ethics Committee of the Faculty of Medicine, Maranatha Christian University-Immanuel Hospital Bandung with Decree No: 027 / KEP / IV / 2016.

RESULTS

This study based on the flow of test preparation according to the OECD 2001, which classification of criteria as list in Table 1. OECD criteria (2001) are used for the determination of acute toxicity categories of chemicals as well as for labeling them.

The results showed that LD50 value of all the test materials, single extract of EEDS, EEJB and their combination (ratio 1: 2) was above 2,000 mg/kgBW, because at that doses did not show symptoms of toxicity, organ macroscopic changes, or mortality in mice. So that all the test preparations are classified into Category 5 / unclassified.[12]

Table 1. Criteria for Classification of Test Products according to OECD (in mice) [13]

Dose (mg/kgBW)	Death	Category
5	≥ 2 from 5 mice dead	1
5	≥1 mice showing symptoms of toxicity but no mice dead	2
50	≥ 2 from 5 mice dead	3
300	≥ 1 mice showing symptoms of toxicity but no mice dead	4
300	≥ 2 from 5 mice dead	4
2000	≥ 1 mice showing symptoms of toxicity but no mice dead	5
2000	There are no symptoms of toxicity	5/ *unclassified*

The results of observing the behavior of mice for 24 hours are shown in Table 2

The observation of behavior in the negative control group when the mice were placed on the platform; At T0, total amount of mice which looking upward and downward was the highest, while at T24 there were no mice looking upward, but there werewhich looking downward with an average of 1.2 and the amount were not as much as at T0. In the combination group of EEDS and EEJB, at T0 and T4 looking upward was the highest, while at T24 there were no mice looking upward, but there werewhich looking downward with an average of 0.4. Motoric activity in the negative control group was generally normal, however, there were two mice (40%) showed decrease in motoric activity at T1 / 2 and T1. In the EEDS and EEJB combination group, one mouse (20%) showed decrease in motoric activity at T1 / 2, T4, and T24.

Observations of other behaviors such as hanging, retablishment, flexion, Haffner response, pineal reflexes and cornea result were positive; and negative results on straub observation, piloerection, ptosis, lacrimation, catalepsy, salivation, seizures, tremors, writhing, and vocalization in all mice in both groups, negative and treatment groups, indicating that all mice were normal. During 24 hours observation there were no toxicity or death symptoms in all experimental animals. All observations of toxicity effects were performed on the platform for 2 minutes except for hanging and retablishment.

In macropathological observations, no organ abnormalities found macroscopically. Furthermore, 8 major mouse organs, heart, lungs, liver, spleen, kidney, adrenal, ovaries and uterus were weighed and the weights of OW and IO can be seen in Table 3.

Table 2 Observational Percentage of Mice Behavior during the First 24 hours of Negative Control, EEDS, EEJB and Combinations of EEDS / EEJB

Observe Effects / hours			Negative Control Group						EEDS Group						EEJB Group						EEDS/EEJB Group					
			0	1/2	1	2	4	24	0	1/2	1	2	4	24	0	1/2	1	2	4	24	0	1/2	1	2	4	24
Platform		Up	2.8	0.4	0.6	1	1.2	0	3.4	0	0.2	0	3	0.8	2.2	0.2	0	7.4	1	0.8	4.6	0.6	0.8	1.6	4.4	0
		Down	3.8	0.2	0.4	0	1.8	1.2	4.4	3.6	1	3.4	1.2	1.2	2.6	1.4	3.2	0.4	1.8	1.6	3	1.4	1.6	2.2	1.4	0.4
Motoric Activity	Up	(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	N	(%)	100	60	60	100	100	100	100	100	80	60	80	80	100	60	60	100	100	80	100	80	100	100	80	80
	Down	(%)	0	40	40	0	0	0	0	0	20	40	20	20	0	40	40	0	0	20	0	20	0	0	20	20
	Still	(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Straub		(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Piloerection		(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ptosis		(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pineal Reflex		(%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Cornea Reflex		(%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Lacrimation		(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Catalepsy		(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Body Attitude	N	(%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	ABN	(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hanging		(%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Retablishment		(%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Flexion		(%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Haffner		(%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Mortality		(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grooming		(%)	20	40	0	0	0	40	20	40	0	0	0	40	20	20	0	0	20	0	20	40	0	20	20	0
Defecation		(%)	80	40	40	20	40	100	80	40	40	20	40	100	100	80	80	60	80	80	60	100	20	60	20	60
Urine		(%)	80	60	40	20	60	60	80	60	40	20	60	60	60	80	80	60	40	40	80	80	60	80	60	100
Breath	Fast	(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	N	(%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	Hard to	(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salivation		(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vocalisation		(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Seizures		(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Writhing		(%)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Notes:
N = Normal
T0 =Observation before treatments
T1 = Observation after 1 hour treatments
T4 = Observation after 4 hours treatments
ABN = Abnormal, Back arching, stomach attached to the platform
T1/2 = Observation after half an hour treatments
T2 = Observation after 2 hours treatments
T24 = Observation after 24 hours treatments

Table 3 Average Results of Weighting of Mice Organ Weight (OW)

Notes	Mean of Organ Weight Mice Control Group (gram)							
	Heart	Lung	Liver	Spleen	Kidney	Adrenal	Ovaries	Uterus
Mean	0.1479	0.2777	1.9327	0.1405	0.3827	0.0358	0.0411	0.1869
SD	0.0214	0.0435	0.2332	0.0270	0.0237	0.0440	0.0149	0.1113
Notes	Mean of Organ Weight Mice EEJB(gram)							
	Heart	Lung	Liver	Spleen	Kidney	Adrenal	Ovaries	Uterus
Mean	0.1488	0.2373	1.9682	0.1311	0.3816	0.0090	0.0357	0.1730
SD	0.0109	0.0600	0.1130	0.0360	0.0156	0.0040	0.0037	0.0763
Notes	Mean of Organ Weight Mice EEDS (gram)							
	Heart	Lung	Liver	Spleen	Kidney	Adrenal	Ovaries	Uterus
Mean	0.1625	0.2110	2.3536	0.2189	0.4073	0.0150	0.0325	**0.1613**
SD	0.0062	0.0389	0.1627	0.0464	0.0309	0.0068	0.0167	**0.0933**
Notes.	Mean of Organ Weight Mice EEDS EEJB (gram)							
	Heart	Lung	Liver	Spleen	Kidney	Adrenal	Ovaries	Uterus
Mean	0.1605	0.2463	2.0128	0.1311	0.3951	0.0150	0.0331	0.2042
SD	0.0256	0.0644	0.2323	0.0331	0.0434	0.0068	0.0083	0.0849

Table 4 Calculation Result of Mice Index Organ

Notes	Index Organsof Control Group Mice							
	Heart	Lung	Liver	Spleen	Kidney	Adrenal	Ovaries	Uterus
Mean	0.4747	0.8897	6.2061	0.4520	1.2271	0.1191	0.1304	0.5849
SD	0.0666	0.1218	0.7450	0.0914	0.0419	0.1536	0.0417	0.3098
Notes	Index Organsof EEJB Group Mice							
	Heart	Lung	Liver	Spleen	Kidney	Adrenal	Ovaries	Uterus
Mean	0.4497	0.7120	5.9486	0.3940	1.1535	0.0275	0.1084	0.5324
SD	0.0413	0.1596	0.4748	0.0993	0.0847	0.0128	0.0170	0.2614
Notes	Index Organsof EEDS Group Mice							
	Heart	Lung	Liver	Spleen	Kidney	Adrenal	Ovaries	Uterus
Mean	0.4940	0.6418	7.1410	0.6678	1.2349	0.0462	0.0982	0.4893
SD	0.0347	0.1313	0.3886	0.1577	0.0573	0.0219	0.0480	0.2899
Notes.	Index Organs of Combination Group of EEDS/EEJB Mice							
	Heart	Lung	Liver	Spleen	Kidney	Adrenal	Ovaries	Uterus
Mean	0.5267	0.8186	6.6105	0.4344	1.3016	0.0502	0.1092	0.6693
SD	0.0706	0.2559	0.5302	0.1203	0.1483	0.0238	0.0267	0.2599

Furthermore, index of organs or relative weight were calculated by the formula absolute organ weight divided by body weight and obtained the results of relative weight or index of organs. The results can be seen in Table 4.

Furthermore, OW and IO each were analyzed statistically using unpaired t test. The result of statistical analysis of t test of organ weight (OW) and index organ (IO) between normal group and test material can be seen in Table 5.

The unpaired t test results showed no significantly difference in BO and IO of 8 major organs with $\alpha = 0.05$ between the negative control group and the EEDS, EEJB-treated single group and EEDS/EEJB combination of 2,000 mg/kgBW as shown in Table 5. The results of OW and IO analysis of the lung, liver and spleen organ of the group treated with EEDS were significantly different from the control group (p value <0.05), where weight lung organ was lighter, while the liver and spleen organ were heavier than control.

The largest OW and IO in all groups of mice in this study were liver while the lowest was OW and IO of adrenal.

DISCUSSION

Previous studies shown that both extracts contains similar secondary metabolites: phenolic, flavonoids, triterpenoids, tannins and quinones, while EEDS contains additional steroids, and saponins. The dose ratio between EEDS and EEJB that showing the best effect in losing weight is 1 to 2. [12]

Table 5 Statistical Analysis The t test of the Organ Weight (OW) and the Organ Index (OI) between Negative Control (Normal) Group and Treatment Groups

	Group	Signifi cancy	Con clusion	Group	Signifi cancy	Con clusion	Group	Significancy	Con clusion
Heart	normal	OW 0.182	ns	normal	OW 0.937	ns	normal	OW 0.495	ns
	DS	IO 0.208	ns	JB	IO 0.937	ns	DSJB	IO 0.499	ns
Lung	normal	OW 0.034	*	normal	OW 0.257	ns	normal	OW 0.083	ns
	DS	IO 0.034	*	JB	IO 0.261	ns	DSJB	IO 0.086	ns
Liver	normal	OW 0.011	*	normal	OW 0.767	ns	normal	OW 0.533	ns
	DS	IO 0.013	*	JB	IO 0.770	ns	DSJB	IO 0.536	ns
Spleen	normal	OW 0.011	*	normal	OW 0.652	ns	normal	OW 0.365	ns
	DS	IO 0.015	*	JB	IO 0.653	ns	DSJB	IO 0.365	ns
Kidney	normal	OW 0.195	ns	normal	OW 0.932	ns	normal	OW 0.120	ns
	DS	IO 0.197	ns	JB	IO 0.932	ns	DSJB	IO 0.133	ns
Adrenal	normal	OW 0.327	ns	normal	OW 0.211	ns	normal	OW 0.220	ns
	DS	IO 0.353	ns	JB	IO 0.245	ns	DSJB	IO 0.254	ns
Ovaries	normal	OW 0.415	ns	normal	OW 0.454	ns	normal	OW 0.307	ns
	DS	IO 0.415	ns	JB	IO 0.471	ns	DSJB	IO 0.323	ns
Uterus	normal	OW 0.704	ns	normal	OW 0.824	ns	normal	OW 0.779	ns
	DS	IO 0.704	ns	JB	IO 0.824	ns	DSJB	IO 0.779	ns

ns: no significant ($p > 0,05$)

*: significant ($p < 0,05$)

This study showed that single extract of EEDS, EEJB and combination of both extracts with ratio of 1: 2 doses of 2.000 mg/kgBW did not cause any toxicity symptoms, nor mortality in mice; therefore, the LD50 values of EEDS, EEJB and combinations were assumed above 2,000 mg/kgBW; so all of these test preparations are classified as 5/unclassified categories, which means 90 per cent or more survive without signs of toxicity.[13,14] While another research, Che study, which performed Acute toxicity test of FCD, a soybean extract combined with L-carnitine, in Sprague-Dawley rats reported that no-observed-adverse-effect-level (NOAEL) of FCD was considered to be 2000 mg/kg for male and 1000 mg/kg for female SD rats.[15] Results of Iswantini study, LC_{50} value of ethanol extracts of *Guazuma ulmifolia* toxicity test were more than 1,000 ppm, 1070.93 ppm.[16]

Both extracts, EEDS and EEJB have some similar secondary metabolite content. The results of this study support the results of previous study, namely, Jati Belanda alcohol extract of the acute toxicity test on male Wistar mice has an oral LD50 value greater than 6,324.14 mg/kgBW including the non-toxic practice category[10], whereas the oral LD50 value of black soy extract on the acute toxicity test performed on Sprague-Dawley mice and C57BL / 6 mice was greater than 2,500 mg/kgBW.[14] This was suggests that the ingredients contained in EEDS and EEJB did not cause toxicity symptoms at very high doses.

The observation on the motoric behavior of the mice, i.e. upward and downwardly in all groups have the same behaviour in upward and downward activity at T0; may be due to all mice still adapt in new environments, when the mice were placed for first times on the platform. In addition, downward activity behavior is also to assess the awareness of the mice to the environment because if the mice fell due to not looking down (mice did not look out for observing the circumstances), then it can be said that there was a disturbance in the nervous system (decline of vigilance level) in that mice.[17] At T24, no mice ever looked up this might be because the mice had been able to adapt to the environment, but there was still a downward look that indicated the level of alertness of the mice was still good.

The results of macroscopic examination of organs after single-dose EEDS, EEJB and combinations administration showed normal results, except the lung, liver and spleen organ of the EEDS group, however further studies were required by microscopic examination to proof this fact.

The OW and IO of the liver had the greatest value among the 8 organs examined. The liver is a complex organ and has many functions, including the excretion and synthesis of bile, cholesterol, glucose, and protein. Other major functions are metabolism of drugs, proteins, fats, steroids; hematopoiesis, storing fat reservoir, glycogen, vitamins, and iron;

phagocytosis of foreign matter, and detoxification and conjugation of toxins in the body are also included in liver function.[18]

The liver organ weight of a mouse is influenced by genetic factors.[16] In this study the weight of liver in the control and treatment groups varied greatly. In EEJB, the dominant secondary metabolite is tannins[17] and the tannin-active substance is tannate acid which can cause liver damage and its symptoms are affected by duration of exposure and depends on its concentration.[18] Large amounts of tannate may cause irritation. Symptoms that may arise include gastric irritation, nausea, vomiting, liver damage, and prolonged use may aggravate liver damage.[19]

Compared to the negative control groups t-test results of the lung, liver, and spleen index of the EEDS 2,000 mg/kgBW combination group showed significantly differences (p value > 0.05). The weight of lung organ in the treatment group were lighter than the control group, so it was assumed that there was no inflammatory process such as hypertrophy or hyperplasia of organ cells resulting from the provision of the test material, therefore the lighter weight of the lung organ can be neglected.

Liver and spleen weights in treatment groups were heavier than the control, it becomes questionable whether the test substances caused inflammation. In most cases Herbs Induced Liver Injury (HILI), inflammation occurs in a therapeutic dose of suggested herbs.[20] This caused a small amount of toxic metabolites in the liver that were not detectable in the blood as a diagnostic marker. In cases of intrinsic type of liver inflammation, which depends on doses and is caused by measurable compounds, very different conditions occur. In this condition liver function tests is absolutely required. Toxic hepatic sinusoidal obstruction syndrome can be caused by plants that contain unsaturated pyrolizidine alkaloids (PAs).[20] From the examination of secondary metabolites it is known that EEDS did not contain alkaloid[17], so the possibility of the addition in liver weight was not caused by a sinusoidal obstruction due to toxic materials. Supported by the results of previous studies, subchronic toxicity tests, extract of combination of Detam 1 soybean and jati Belanda for 90 days and observation for 120 days in mice Wistar male and female, showed a good effect on liver function, did not cause severe alterations to liver weight and at a dose of 150 mg/KgBW did not cause histopathological disorders of the liver.[21]

Other studies showed that a single effect of EEDS led to an improvement in histopathological features of the liver.[6] The likelihood of increased liver weight to the experimental animals is that the organ has been damaged before the study, but it was difficult to ascertain because the liver function was not measured before the study begin. This was a limitation of this study. The result of unpaired t-test of the group given single extract of Jati Belanda which was estimated to have a bad effect on liver organ, evidently showed not significantly difference with the negative control, as well as the provision of combination test materials, so it was concluded that single EEJB preparation and combination of both extract (1 : 2) dose of 2,000 mg / kgBW orally did not affect the weight (WO) and index (IO) of the liver.

The spleen is a ductless gland organ that is closely related to the circulatory system and serves as destroyer for the aged and damaged red blood cell destroyer. Another function of the spleen is to prevent infection and act as the first defense against invasive pathogens.[22] Spleen includes one organ of the lymphoid system, in addition to the thymus, tonsils and lymph nodes. Lymphoid system serves to protect the body from damage caused by foreign substances.[23] Swelling of the spleen are generally no symptoms, called an inflamed spleen splenitis, usually occurs in the red part of the spleen, the cause could be a wide range, one of which is caused by exposure to chemicals or radiation. Pathologic enlarged spleen can occur due to inflammation and can be acute, chronic, granulomatous, or abses.[24,25] Sub-chronic toxicity test results with the combination of Detam 1 soybean and Jati Belanda extracts for 90 days and observation for 120 days, showed that, the weight of all the vital organs of male Wistar rats, included adrenal, kidney, liver, heart, spleen, lung, testis, and vesicalis seminal, were not significantly difference compared to the negative control group (p value= 0.228. Spleen organ of female Wistar rats showed significantly difference to the satellite control group (p value <0.05) while the weight of the spleen in the treatment groups were lower than the negative control.[256] The possibility of spleen weight increased in experimental animals in this research is that there was already impaired organ before the research begin.

In this study, adrenal glands in all groups had the smallest OW and IO among the other 8 organs examined. T- test analysis on OW and IO of the adrenal glands between negative control with all of treatment groups showed non significantly difference (p value > 0.05). Adrenal gland is an endocrine organ that has many physiological functions such as metabolism, stress response, immune function, and

has a role in cardiovascular function.[27] The growth of adrenal gland is affected by age and sex, in which the adrenal gland growth in female mice is twice as likely as the male mice at 9 weeks of age and adrenal gland growth of female mice reached their peak at 7 weeks.[27] Female mice aged 8 week at the start of the experiment were used in this study, which means that the adrenal glands weight were already stable as described above. The OW and IO of the adrenal glands in all treatment groups were varied, however, they were statistically no significant difference, therefore it can be concluded that the administration of all treatment substances per oral did not affect the weight and index of the adrenal glands and also the macroscopic pictures.

In conclusion, the LD50 value of Detam 1 Soybean ethanol extract (EEDS), Jati Belanda leaf ethanol extract (EEJB) and the combination of both with 1: 2 ratio in the acute toxicity test were within a wide margin of safety, above 2,000 mg/kgBW and classified into the category 5 / unclassified (OECD, 2001) because there was no death in all experimental animals and the effect on Swiss Webster's mice behavior, weight and index of organs on all test materials was not different from the negative control, except the weight and index of liver and spleen organ EEDS.

Acknowledgments

We would like to thank the Director of Research and Community Service of the Directorate General for Research and Development of the Ministry of Research, Technology and Higher Education of Indonesia for the Grant of Research on Applied Product Grant No.105/ SP2H/PPM/DRPM/II/ 2016 dated February 17, 2016, so that this research can be done.

REFERENCES

1. Katno. Tingkat manfaat keamanan dan efektivitas tanaman obat dan obat tradisional. Tawangmangu: B2P2TO-OT Depkes RI. 2008. Indonesian.
2. Pramono E. The commercial use of traditional knowledge and medicinal plants in Indonesia. Multi-stakeholder dialogue on trade, intellectual property and biological resources in Asia. 2002.
3. Sulaksana J. Kemuning dan Jati belanda dan pemanfaat untuk obat. Jakarta: Penebar Swadaya. 2005. Indonesian.
4. Adisarwanto T. Kedelai. Jakarta: Penebar Swadaya. 2005. Indonesian.
5. Hidayat M, Soeng S, Prahastuti S, Kwan M, Krisetya YA, Renadia N. Combination effects of Ethanol extracts Detam m1 Soybean and Jati belanda leaves to body weight, cholesterol and Triglyceride in male Wistar rats. Journal of The Indonesian Medical Association. 2014;64(8): 372-6
6. Hidayat M, Soeng S, Wahyudianingsih R, Ladi JE, Krisetya YA, Elviora V. 2015. Ekstrak kedelai Detam 1, daun Jati belanda serta kombinasinya terhadap berat badan dan Histopatologis hati mencit Wistar. JKKI, 4(6):167-78.
7. Yalniz M, Bahcecioglu IH, Kuzu N, Poyrazoglu OK, Bulmus O, Celebi S, *et al*. Preventive role of Genistein in an experimental non-alcoholic Steatohepatitis model J Gastroenterol hepatol. 2009;(22): -14.
8. Anonim. Pedoman pelaksanaan uji klinik obat tradisional: Tata laksana uji praklinik obat tradisional: Tata laksana teknologi farmasi obat tradisional: Tata laksana uji klinik obat tradisional. Edisi 1. Jakarta: Departemen Kesehatan RI. 2000. Indonesian.
9. Badan Pengawas Obat dan Makanan Republik Indonesia. Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia Nomor 7 Tahun 2014 tentang Pedoman Uji Toksisitas Nonklinik secara In Vivo.Jakarta:BPOM; 2014.
10. Utomo AW. Uji toksisitas akut ekstrak alkohol daun Jati belanda *(Guazuma ulmifolia Lamk)* pada mencit Wistar. Universitas Diponegoro Semarang: Karya Tulis Ilmiah. 2008.
11. Hidayat M, Prahastuti S, Chikita V, Safitri D, Rahmawati SF, Soemardji AA. Pemberian subkronis kombinasi ekstrak kedelai Detam 1 dan Jati belanda tidak berefek toksik terhadap fungsi, berat, dan Histopatologis ginjal tikus Wistar. Journal of Medicine and Health. 2016; 1(4): 341-50.
12. Hidayat M, Soeng S, Prahastuti S, Tiono H, Krisetya YA, Sugiono M. Characteristics of Ethanol extract of Detam 1 Indonesian soybean, Jati belanda leaves and the effets of their combinations to weight gain and the Jejunum histopathological changes in male Wistar rats. European Journal of Medicinal Plants. EJMP.2015:7(2):87-98.ISSN: 2231-0894.
13. Organization for Economic Co-operation and Development. OECD guidelines for testing of Chemicals, 401, 407-408, 420. OECD. 2001.
14. Fukuda I, Tsutsui M, Yoshida T, Toda T, Tsuda T, Ashida T. Oral toxicological studies of black Soybean *(Glycine Max)* hull extract: Acute studies in rats and mice, and chronic studies in mice. Food Chem Toxicol. 2011;49(12):3272-8.
15. Che JH, Kwon E, Kim SH, You JR, Kim BH, Lee SJ, et al. Acute and subchronic toxicity of FCD, a soybean extract combined with L-carnitine, in Sprague-Dawley rats. Regul Toxicol Pharmacol. 2011;59(2):285-92. doi: 10.1016/j.yrtph.2010.11.001.
16. Iswantini D, Silitonga RF, Martatilova E, Kadarusman LK. Zingiber cassumunar, Guazuma ulmifolia, and Murraya paniculata Extracts as Antiobesity: In Vitro Inhibitory Effect on Pancreatic Lipase Activity. Hayati Journal of Biosciences. 2011;18(1): 6-10
17. Institutional Animal Care and Use Committee (IACUC). Weight Loss in Research Animals. 2014.

18. Jones LD, Nielsen MK, Britton RA. Genetic variation liver mass, body mass, and liver: Body mass in mice department of animal science. University of Nebraska, Lincoln. 1992. pp 3000-6.
19. Bayupurnama. Hepatotoksisitas imbas obat. Buku ajar ilmu penyakit dalam. Edisi 4. Jakarta: Pusat Penerbitan Departemen Ilmu Penyakit Dalam FKUI. 2006.
20. Danan Gand Teschke R. RUCAM in Drug and Herb Induced Liver Injury: The Update Int JMolSci.2016;17(1):14.Availablefrom:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4730261/
21. Hidayat M, Prahastuti S, Dewi E, Safitri D, Soemardji AA. Subchronic toxicity test of combination Ethanol extract of Detam 1 Soybean (*Glycine max* L.Merr) and Jati belanda leaves (*Guazuma ulmifolia* LamK) toward function, weight and Histopathological wistar rats liver. AJPCR. Asian Journal of Pharmaceutical and Clinical Research. 2016;9(6):197-201.
22. Badr FM. In vivo secretion of 11-Deoxycortisol by the mouse adrenals: Plasma corticosteroid levels in three strains of normal mice. Comp Biochem Physiol B. 1971;(39): 131-7.
23. Aughey E, Frye FL. Comparative veterinary histology with clinical correlates. London: Iowa State University Press. 2001. p 215-26, 250-1.
24. Junquereira LC, Carneiro J. Basic Histology. Dharma A, translator. Jakarta:EGC.1982.p 287-308, 323-35.
25. Ward JM, Mann PC, Morishima H, Frith CH, Thymus, Spleen, et al. In: Maronpot RR, GA Boorman, BW Gaul, Editor. Pathology of the mouse reference and atlas. Vienna: Cache River Press. 1999. p 333-57.
26. Activity of Soybean etanol extract (*Glycine Max* L.Merr) Detam 1 Varieties, Jati belanda leaves (*Guazuma Ulmifolia*) and combination as antiobesity through Pancreatic lipase Enzyme inhibition 2nd Year: Subchronic toxicity test combination Ethanol extract of Detam 1 Soybean seeds (EEDS) and Ethanol extract of Jati belanda (EEJB) in In vivo on wistar rats for 90 and 120 Days. Competitive Grant Report. DIKTI.2015
27. Bielohuby M. Growth analysis of the mouse adrenal gland from weaning to adulthood: time-and gender-dependent alterations of cell size and number in the cortical compartment. Am J Physiol Endocrinol Metab. 2007. p 10-39.