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Acute Toxicity Of Soybean Extract With Targeted Lunasin (ET-Lun)

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Abstract. ET-Lun is an extract containing Lunasin as an active compound. Lunasin was extracted using PBS with pH 7,4 from defatted soybean powder. Several studies proved ET-Lun could reduce the expression of COX-2 and iNOS. ET-Lun can inhibit angiogenesis, increase apoptosis and reduce dysplasia. ET-Lun might also decrease EGFR expression in DMBA induced breast cancer rats. The aim of this study was to evaluate the acute toxicity of ET-Lun using Sprague Dawley (SD) rats. Two groups (n = 10) were orally given a single dose of ET-Lun at 2000 mg/kg and 5000 mg/kg weight. The control group (n=5) received only vehicle distilled water. In the end, the rats were sacrificed. The blood was collected for hematological evaluation and liver and kidney histopathology was examined afterward. There were no toxic signs on the administration of ET-lun doses of 2000 and 5000 mg/kg . The histopathology of the liver and kidney groups showed no difference between the treatment group and the control group. Furthermore, creatinine, urea and AST and ALT levels shown no difference between the treatment group and control ($p > 0.05$). ET-Lun has LD50 more than 5000 mg/kg BW and practically non-toxic.

Keywords: soybean, acute toxicity, liver, kidney, LD50.

INTRODUCTION

Soybean (Glycine max (L.) Merr.) is one of plant that has been being explored for its medicinal activity. Many studies have been carried out to develop the pharmacological activity of active compounds from soy, including anticancer, osteoporosis, cognitive disorders, cardiovascular disease, and kidney function disorders [1]. One of the active compounds from soy that has been developed as medicine is lunasin [2].

Lunasin, a 43 amino acid polypeptide, has 3 domains were the tail aspartic acid domain, the Arg-Gly-Asp (RGD) domain, and the helical chromatin-binding domain. These domains reported, have pharmacology activity [3]. However, lunasin synthesis was expensive while methods to purified pure lunasin from plants was still limited. More importantly, both lunasin synthesis and purifications takes a long time [4,5]. Analysis of different soybean cultivars shown that the lunasin content also varies significantly [6]. To overcome these problems was by produced ET-Lun. ET-Lun is a crude extract, contain lunasin that was extracted using PBS with pH 7,4 from defatted soybean powder [7].

Some studies that have been done regarding ET-Lun activity were; ET-Lun can significantly reduced COX-2 and iNOS expression [8]. Furthermore, ET-Lun has activity to suppresed of Goblet cell count and microvascular density [9], increasing apoptosis and reduced dysplasia [10]. ET-Lun might also decrease EGFR expression in DMBA induced breast cancer rats [11].

To determine the safety of ET-Lun as a candidate of medicine, it is necessary to conduct a toxicity test in an animals model to investigate further biochemical, physiological, and pathological reactions in humans to the test preparation. Acute toxicity test is a test of the toxicity of a compound given in a single dose to experimental animals, which is observed for 7-14 days. In this study, the observations were to determine the Lethal dose of 50% of experimental animals (LD50) and analyzed liver and kidney function by aspartate aminotransferase (AST) or serum glutamic-oxaloacetic transaminase (SGOT), alanine aminotransferase (ALT) or serum glutamic pyruvic transaminase (SGPT), creatinine serum, urea levels, and their histology [10]. The purpose of this study was to evaluate the safety of the ET-Lun test preparation by conducted an acute toxicity study.

MATERIALS AND METHODS

Plant Extraction and Extract Standardization

The extract was made by using the maceration method. The simplicia powder of Soybean seeds that has been defatted was put into a macerator that is protected from sunlight, followed by extraction with a PBS solution of pH 7.4 for 60 minutes [7]. Maserate separated using filter paper. All the macerate that has been collected was then evaporated using a rotary vacuum evaporator at a temperature of 45°-50° C until a thick extract was obtained. The thick extract obtained was then tested for extract standardization.

Acute Toxicity Study

The 15 female rats were divided into 3 groups, each group consisted of 5 female Sprague Dawley (SD) rats aged within 8-10 weeks. Group I was a normal control. Group II, III were the treatment groups given ET-Lun at 2000 and 5000 mg/kg weight, respectively. ET-Lun was given in a single dose and observations were made for 14 days. Before being given ET-Lun, the rats have fasted for 16 hours. The study was performed and having approval by the Ethics Commission of the Faculty of Medicine, the University of Indonesia (the numbered certificate was KET-647 / UN2.F1 / ETIK/ PPM.00.02 / 2019.

Hematology Examination

Rat blood serum was used for blood chemistry tests; the level of creatinine, urea, ALT, and AST.

Histopathological Evaluation

Rats from each group were sacrificed at the end of the treatment by injection of ketamine at a dose of 75-100 mg / Kg BW by IP and xylazine at a dose of 10 mg / Kg BW by IP. Then the mice were dissected to take the liver and kidneys, and testes. The organs were then put in a 10% formaldehyde buffer (NBF) solution and made histopathological preparations using HE (Hematoxylin Eosin) staining.

Data Analysis

The normality data of the level of creatinine, urea, ALT, and AST were evaluated using Kolmogorov-Smirnov and homogeneity by Levene. Data analysis was performed using ANOVA and Tukey test. The data presented as mean + SD. The results were considered significant if the p-value <0.05.

RESULTS AND DISCUSSIONS

Acute Toxicity Study

Acute toxicity test is a method designed to determine the lethal dose mean (LD50) of a certain compound [12]. LD50 was defined as dose or concentration from a certain compound that is given a single or multiple times within 24 hours, which statistically expect to kills 50 % of animal models [13]. This study used 15 female SD mice which consisted of a normal group, a group with a dose of 2000 and 5000 mg/kg BW respectively. Each group consisted of 5 female SD rats.

Doses were determined using fixed doses methods which refer to the National Agency of Drug and Food Control (NADFC) Indonesia [14]. Early doses were chosen by referring to preliminary tests to identify doses to produced symptoms of mild toxicity without the development of heavy toxicity to death. This procedure was proceeded to a sufficient dose until the toxic effect or not cause more than 1 death, or the toxic effect was not found until the highest dose or death observed in the group with lower doses.

ET-Lun administration of 2000 mg/kg and 5000 mg/kg BW single doses did not cause death and toxicity signs within 24 hours or 14 days of observation. There were not toxic symptoms and no change in behavior such as weakness, seizures, excessive diarrhea, and change in stool or urine color. Experimental animals were active and can respond the stimuli such as touching (**TABLE 1**).

TABLE 1. The Observation of toxicity signs in 24 hours and 14 days

Group of treatment	Signs of toxicity	
Normal	The symptoms	There was no change in the color of the stool or urine, there was no excess diarrhea
	The Behaviors	No convulsions and weakness
eatment group doses 2000 mg/kg BW and 5000 mg/kg	The symptoms	There were no changes in the color of the stool or urine and no diarrhea
	The Behaviors	No spasm and illness

The result of this study showed LD50 of ET-Lun was more than 5000 mg/kg. Its means the ET-Lun was classified to be practically non-toxic [13]. This study was supported by other studies that prove the oral LD50 of soy ethanolic extract was more than 2000 mg/kg [15].

The mean eight of the kidney was 1,74±0,05 grams in the control groups; 1,8±0,16 grams and 1,88±0,04 grams at 2000 and 5000 mg/kg BW. There was no significant difference in the weight of the kidney betwee he control and treatment groups (p> 0.05). Furth more, the liver weight of control group was 8,38±0,43 grams; the dose of 2000 mg/kg was 8,96±0,56 grams, and the dose of 5000 mg/kg was 8,42±0,26. The data showed that there was no difference in liver weight between the normal group and the treatment group (p> 0.05).

To analyze the toxicity of drugs or plant materials, most researchers used liver and kidneys organs. The liver plays an important role in metabolism, while the kidneys play a pivotal role in excretion of medicine [16,17].

Hematology Examination

The Parameter preference of AST and ALT was due to those were aminotransferase enzymes that are sensitive indicators of liver organs damages. More over, creatinine and urea levels are sensitive indicators of renal function [16]. The results of creatinine and urea examination from rat serum shown that there was no difference in serum creatinine and urea levels in treated rats and normal controls (p> 0.05). The creatinine level (**Fig. 1a**) in normal control was 0.52 mg/dl. In the treatment groups doses of 2000 mg/kg was 0.606 mg/dl, and doses of 5000 mg/kg was 0.66 mg/dl. The urea level was 17.72 mg/dl in the control and treatment groups dose of 2000 mg/kg was 18.52 mg/dl, and the group dose of 5000 mg/kg was 18.5 mg/dl (**Fig. 1b**).

Urea and creatinine are the main indicators of kidney damage.[16] Creatinin was metabolite and totally excreted to urines by glomerulus filtration. Therefore, an increase in the level of creatinine indicated that kidney functions were damaged. Creatinin is the most critical function of the kidney is to eliminate the potentially toxic substances from the body. Urea was the end product of protein and amino acid metabolism that contained nitrogen. Increase blood urea nitrogen may be due to decrease glomerulus filtrations shown disturbance of kidney functions [16,17].

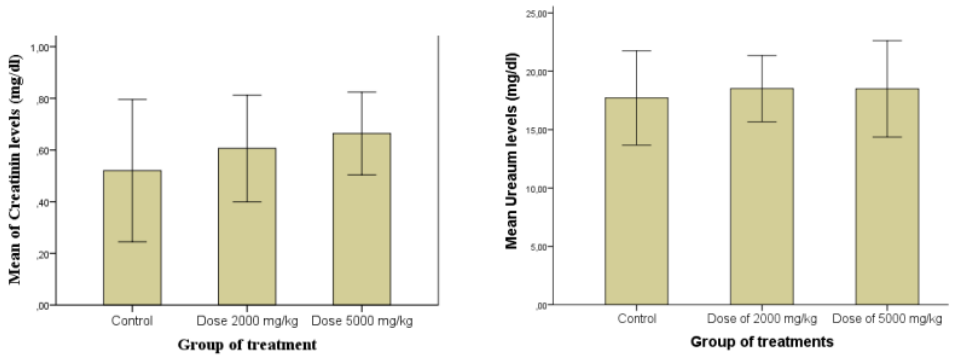

FIGURE 1. The creatinine and ureum level of the treatment group. Data are presented as means±SD (n=5). *p<0,05

The means level of AST in control and treatment groups showed no difference statistically ($p > 0.05$) (**Fig. 2a**). Moreover, the ALT levels (**Fig. 2b**) in controls and treatment groups also showed no difference significantly ($p > 0.05$).

The AST was a mitochondrial enzyme that was frequently found in the heart, liver, muscle, and kidney while ALT was frequently found in several tissues even the main source is the liver. In normal or healthy conditions, intracellular enzymes such as AST and ALT were found in normal ranges. Increased level of AST and ALT in blood indicates certain organ damage, including the liver. The mechanism behind this was when liver cells are damaged, the AST and ALT were released from cells and enters circulations [18].

AST and ALT were transaminase enzymes that play role in enzymatic catalysis reactions in protein metabolism. ALT was higher in the kidney and AST can find in the heart, skeletal muscles, and liver. When the hepatocyte was damaged, ALT and AST will be released into the serum, and increased in serum [19].

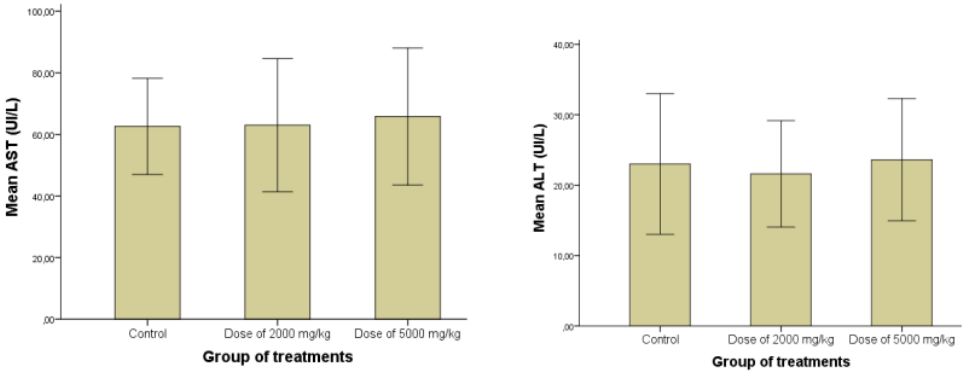

FIGURE 2. The AST and ALT level (UI/L) of the treatment group. Data are presented as means±SD (n=5). *p<0,05

Histopathological evaluation

The hematology examination result was also supported by a histopathological study of the liver and kidney. The histopathological studies showed no significant difference between the treatment and controls groups..

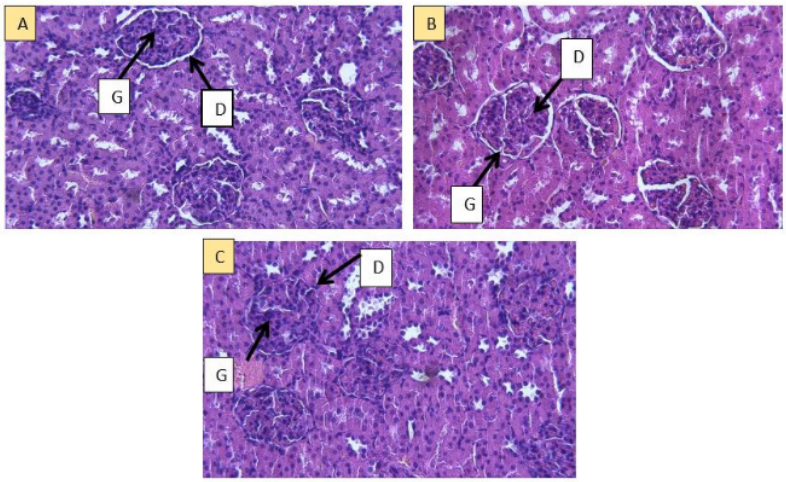

FIGURE 3. Histology Profile of Kidney Tissue with Haematoxylin-Eosin Staining, 400x magnification. A=Normal Group, B=ET-Lun group dose 2000 mg /kg, C=ET-Lun group dose of 5000 mg/kg BW. G=Glomerulus, D=distance between the glomerulus and bowman's capsule

The microscopic examination showed no damage to the kidney; there was no dilatation, hypertrophy, or degeneration of the tubules in control and group treatments. The length between the glomerulus and bowman's

capsule show no distinct compared with the controls. (**Fig. 3**). The results of the liver histology examination in the treatment group showed no liver necrosis and no dilatation of the central veins and sinusoids along with the addition of the dosage of the treatment (**Fig. 4**).

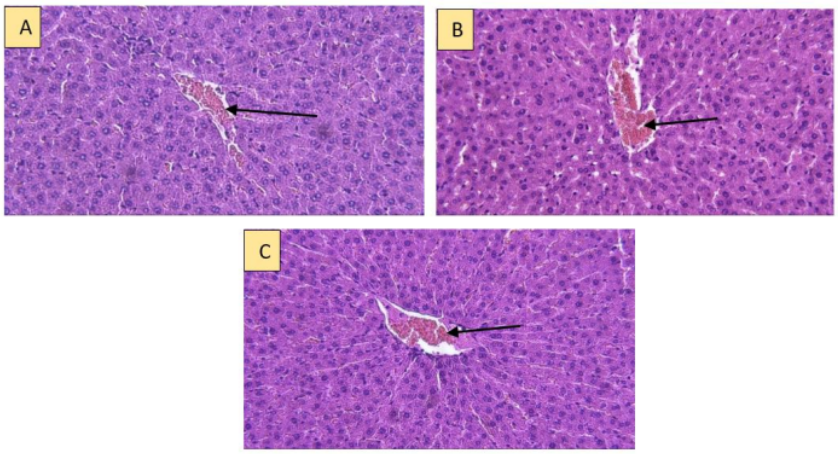

FIGURE 4. Histological Profile of Liver Tissue with Hematoxylin-Eosin Staining at 400x magnification. A. Normal Group. B. ET-Lun group, dose 2000 mg/kg BW. C ET-Lun group dose of 5000 mg/kg BW. The arrow indicates the central vein.

; there was no dilatation, hypertrophy, or degeneration of the tubules in control and group treatments.

CONCLUSION

Acute toxicity study of ET-Lun showed LD50 was greater than 5000 mg/kg BW, and ET-Lun was practically non-toxic. ET-Lun is safe to use as a candidate of herbal medicine and could be continued with subchronic toxicity assay.

COMPETING OF INTEREST

None to declare.

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